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PLANT PHYSIOLOGY

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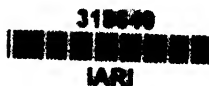
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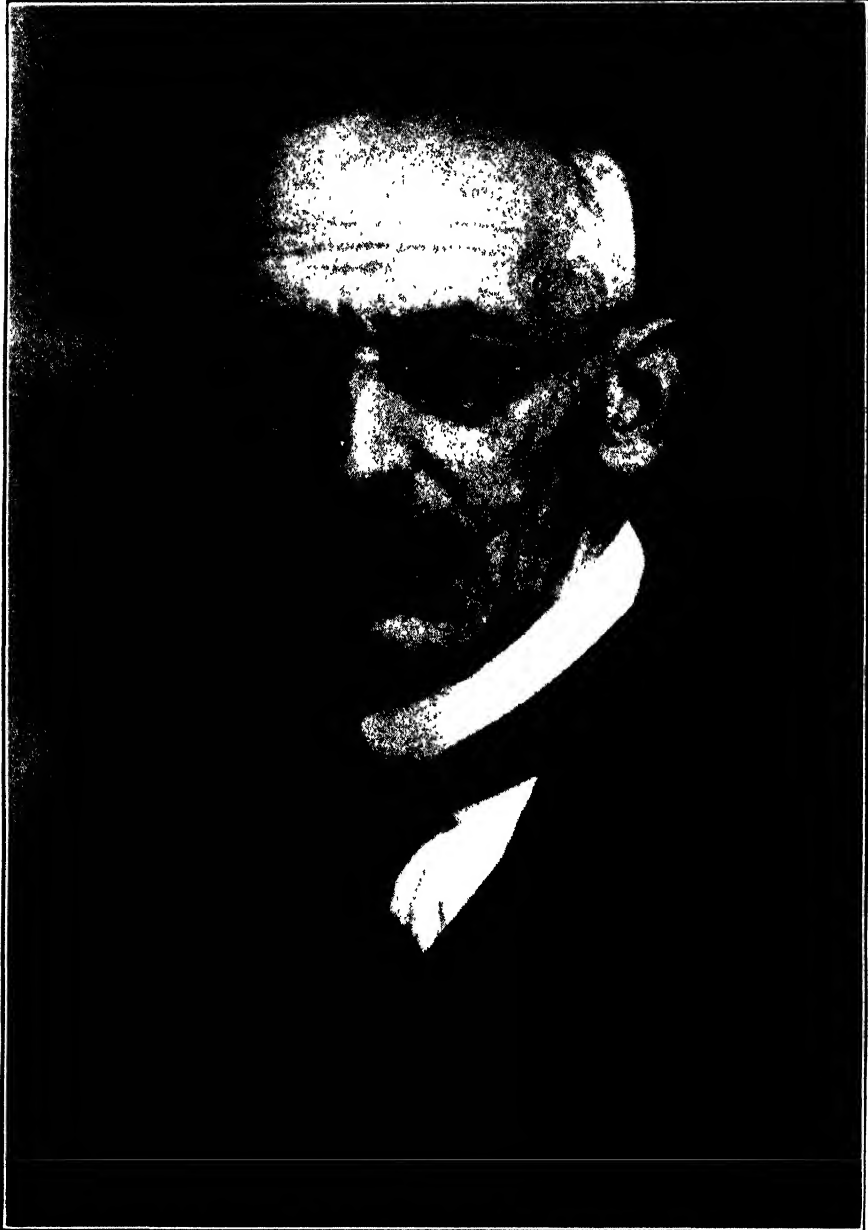
ERRATA

Page 22, table XI, column 4, for 5.06 read 5.08.

Page 23, second line below table XII, for 0.3271 read 0.2371.

Page 82, line 14 from bottom, for bulk read bulb.

Page 84, line 12 from bottom, for raising false stems read raising the false-stems.
Page 85, last line, for SCHWARTZ read SCHWARZ.
Page 92, second line of legend, fig. 13, for 12 read -2.
Page 93, average of -1, A, for 21.7 read 21.1.
Page 95, fig. 14, highest ordinate at left, for 130 read 135.
Page 101, citation no. 5, for SCHWARTZ read SCHWARZ.
Page 152, line 9 from bottom, for standard read saturated.
Page 223, line 17 from bottom, for (16) read 16.
Page 257, title of table IV, for WHITE read WITTE.
Page 258, line 6 from bottom, for are read is.
Page 264, line 3, for Hochaktiver read hochaktiver.
Page 372, line 2, for Linksstrasse read Linkstrasse.
Page 433, line 14, omit a before $\text{Ca}(\text{NO}_3)_2$.
Page 477, line 5 from bottom, for immerson read immersion.
Page 481, first line of footnote, for prophylene read propylene.



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PLANT PHYSIOLOGY

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MOVEMENT OF ORGANIC MATERIALS IN PLANTS

ALDEN S. CRAFTS

(WITH FOUR PLATES AND THREE FIGURES)

Introduction

Many theories have been proposed to explain the movement of foods in the plant but few of them seem sound when critically examined. Most of these theories fail to describe a mechanism capable of conducting the required amounts of material through the available channels with the necessary rapidity. Such a mechanism must exist and it must be compatible with the form and structure of the plant in which it serves.

The first theories on organic transport (42, 27, 39) were based upon ringing experiments but the interpretations of these experiments were so influenced by animal physiology as to render the theories untenable. HARTIG (29) first described phloem tissues and recognized their relation to food conduction. He proposed protoplasmic streaming (30) as an accelerating mechanism in the distribution of these materials and he also observed exudation from the phloem (31).

Following HARTIG a controversy as to which tissues functioned in conduction (28, 49, 58, 32) occupied the attention of investigators. NÄGELI (49) suggested changing turgidity as a cause for movement along sieve-tubes and DE VRIES (17) elaborated the protoplasmic streaming theory, supporting his hypothesis with some microscopical studies.

ZACHARIAS (67) and KRAUS (41) contributed data on the composition of the exudate from the cut phloem of cucurbits, their analyses indicating a high content of food materials. Little work has been done on phloem exudates since that time and the idea, developed by early botanists, that this material flowed from cut sieve-tubes (25, 26) has apparently never been contested. The anatomical studies of HILL (33, 34) and his predecessors (65, 34) and the physiological and morphological investigations of RUHLAND (57) and SCHMIDT (60) seem to justify a reasonable doubt as to

the function of the sieve-tube in conduction. If pores actually exist in the protoplasmic connections of these elements their extreme fineness makes questionable the importance of mass flow.

Ringing has survived as the principal method for studying translocation (16, 37, 44) though at best it can only hint as to the mechanism involved. The experiments of UZAPEK (15), CURTIS (11, 12, 13), and MASON and MASKELL (46, 47, 55) are typical of this method and they indicate quite conclusively that downward movement of organic materials takes place through the bark. The experiments of MASON and MASKELL show that concentration gradients exist in the phloem and that they are more or less related to the rate and direction of movement.

BIRCH-HIRSCHFELD (4) carried out a number of experiments attempting to demonstrate conduction of lithium and dyes through plant tissues. Finding movement through the phloem to be very slow she proposed that downward conduction occurs in the xylem. DIXON (21), and DIXON and BALL (20, 22) have emphasized the inadequacy of sieve-tubes as conducting elements and have further elaborated the mechanism proposed by BIRCH-HIRSCHFELD. Although their calculations in no way prove the impossibility of mass movement through the phloem, they show the necessity for mathematical analysis of the problem and suggest a method for measuring the fitness of any hypothesis.

CURTIS, finding that ringing interferes with the upward movement of nitrogen as well as with the downward movement of organic materials, advocates the protoplasmic streaming hypothesis as providing for movement in both directions through the same tissue at the same time. He gives no calculations as to the rates at which foods are moving but considers acceleration due to streaming to be rapid enough to account for growth and storage. In a late paper (14) he emphasizes the importance of living cells in this mechanism.

Other theories have appeared from time to time (26, 43, 38) but the one proposed by MÜNCH (48) is of the greatest significance in relation to the present work. He described a physical system composed of two osmometer cells containing solutions of different concentrations connected by a tube. When this apparatus was placed in pure water a mass flow took place resulting in a transfer of solute from the cell of higher concentration to the one of lower concentration. He applied this system to the plant assuming that photosynthesis maintains a concentration difference between assimilating cells and storage or meristematic tissues, the sieve-tubes acting as the connecting elements. He described experiments in which exudation from the cambium and excess growth resulted from interruption of the descending sap stream. Cutting the phloem caused a very rapid decrease

in exudation pressure, especially below the cut. This decrease in pressure was transmitted downward at a rate of ten centimeters per second for as far as six meters. MÜNCH calculated the rate of flow necessary to account for certain known increases in dry weight of trees, and estimated that about two and one half per cent. of the total water used by the tree circulates through the phloem system. He suggested that transfer of assimilate from leaf parenchyma to sieve-tubes takes place along the plasmodesma assuming that the property of selective permeability is confined to the outer layer of the protoplast.

Existing theories

The most prominent theories concerning food conduction in plants as described in the literature may be classified under two heads: (1) those favoring movement through the xylem and (2) those advocating the phloem as the channel.

(1) The first of these, as developed by DIXON and BALL, proposes that a solution of organic nutrients having passed from synthesizing cells of the leaf due to increased permeability will be drawn down through the xylem tubes by the water columns in tension and will be absorbed at lower levels by living cells and distributed laterally throughout the stem or root.

There are several serious objections to this mechanism based upon well known or easily demonstrated anatomical facts. In the first place the open junction through which the downward moving xylem sap flows to join the ascending transpiration current must be located at a considerable distance below the point of entry of the organic materials; in fact, for the nutrition of the root tip it must occur at the extreme lower end of the two tubes concerned. This is not the case in the normal plant. A series of experiments on the movement of dyes in leaves has shown that water movement may take place in any direction indicating that the xylem system forms an interconnected network of tubes in which movement is determined by pressure gradients which depend upon size of openings and rate of water loss. It seems therefore that if any particular portion of a leaf became permeable, allowing a flow of solution from the mesophyll cells into the xylem, it is improbable that this solution would move out of the leaf.

That a similar system, though not so closely connected, exists throughout the plant may be shown by cutting petioles and stems under eosin. The solution will flow backward until it meets a junction with another portion of the plant and will then join the ascending stream and finally arrive at a transpiring surface. The only way in which the underground portion of plants could be injected was by allowing the plants to absorb eosin until the transpiring surfaces were all killed, whereupon a complete reversal of flow set in, caused by a water deficit developed in those roots which were

surrounded by dry soil. In no case did the eosin solution move out into the root tips. In fact, in *Convolvulus arvensis*, upon which many of these experiments were made, it is impossible to explain the nutrition of the root tip on the basis of movement through the xylem because the radially arranged primary xylem strands remain isolated (65) from the secondary xylem by unligified living parenchyma cells. In this case the descending stream of nutrient sap could not approach within several centimeters of the rapidly growing tips of main roots without crossing the barrier of living cells. It is difficult to see how it could do this if moved by tension resulting from transpiration. The roots of many woody plants are known to grow when the parts above ground are leafless and dormant. A mechanism dependent upon tension cannot explain nutrition of their growing points. A consideration of the structure of woody tissues lends further weight to this argument. It is a well known fact that lignified cell walls are permeable to water and dissolved substances though fairly resistant to rapid lateral movement (65, 23) so that any imbibition deficit (61) which existed in one portion of the xylem would be transmitted to the surrounding tissues. Then since pressure acts in all directions equally a pressure gradient in the longitudinal direction would also exist laterally, so that a solution flowing downward due to increased pressure in an individual vessel or chain of tracheids would also tend to spread to surrounding elements either through the wall or through pits (2). In tall trees, therefore, where the lowest leaves may be from 50 to 100 meters from root tips it seems highly improbable that longitudinal movement through the xylem could supply organic nutrients to the roots in view of the constant loss from the descending stream by lateral movement. The data presented by CURTIS, MASON and MASKELL and others make the xylem theory even more untenable; their experiments provide convincing evidence for the longitudinal transfer of carbohydrates through the bark.

(2) Of those theories which favor the phloem as the channel for transport, some consider the movement from cell to cell by a process similar to diffusion and possibly accelerated by protoplasmic streaming as the best explanation, others favor a mass flow through the sieve-tubes. The first of these theories is open to serious objection. The parenchyma and companion cells of the phloem are typical living cells with semipermeable protoplasts and are relatively impermeable to sugars. RUHLAND (57) found that he could plasmolyse these cells with hypertonic solutions of sucrose, fructose and glucose and that recovery was slight even after 24 hours. The same results were obtained with cucurbit tissues in the present work. Longitudinal sections of phloem tissue were allowed to absorb neutral red for a few minutes and then mounted in hypertonic sucrose solution. The cells were rapidly plasmolysed and showed no recovery after several hours.

These results indicate that if sugar were moving through the phloem the protoplasts of these cells would offer considerable resistance. It is doubtful if the acceleration due to protoplasmic streaming could account for the rates of movement which have been calculated as necessary. In the first place streaming has not been demonstrated to be universal in its occurrence in these tissues. BIRCH-HIRSCHFELD (4) found no evidence that it accelerated movement in her experiments, and BIERBERG (3) found acceleration to be only from 3 to 4 times that of normal diffusion. DIXON (21) calculated that a 10 per cent. sucrose solution would have to move 50 cm. per hour in order to account for the observed translocation into potato tubers. BIRCH-HIRSCHFELD (4) made similar calculations and MASON and LEWIN (45) calculated a rate of 88 cm. per hour for a 25 per cent. solution through the sieve-tubes of the greater yam. If this were moving through the parenchyma cells, the rate could be reduced to about 34 cm. per hour for a 25 per cent. solution or 88 cm. for a 10 per cent. solution. DE VRIES (17) observed a rate of streaming of from 0.2 to 0.4 mm. per minute in phloem cells. EWART (24) records rates up to 3 mm. per minute in water plants and in the present work a maximum rate of 2 mm. per minute was observed in phloem parenchyma cells of cucurbit stems. The maximum rate of 3 mm. per minute would be 18 cm. per hour which would not suffice even if the streaming cell constituted a perfect machine, and if the diffusion in and out through the cell wall and ectoplasm were neglected. CURTIS (13) sees little difficulty in this diffusion across end walls, terming them "occasional membranes"; but since these cells range from 0.5 mm. to 0.2 mm. or less in length the number traversed must necessarily be 20 or more per centimeter.

If the time required for recovery from plasmolysis in hypertonic sugar solutions is a satisfactory indication of the permeability of the protoplasmic layer for sugar molecules, then it must be granted that diffusion through the ectoplasm into the streaming inner layers at one end of the cell, and the subsequent movement out at the other end would be a very slow process. This hindrance, in addition to the time required for diffusion through the walls, would undoubtedly reduce the average rate to one incommensurate with that mentioned by DIXON. Finally, an estimate of the percentage of the total cross-sectional area of the phloem actually occupied at any one time by sugar moving in the proper direction, suggests that a concentration factor of serious magnitude is involved.

Considering next the possibility of a mass flow through the sieve-tubes we find that the narrowness of the pores through the sieve-plates constitutes a serious difficulty. This objection was apparently neglected by NÄGELI (49), SACHS (58), CZAPEK (15), and other early workers. EWART (24) calculates that half an atmosphere would be sufficient to produce a mass

movement of 5 mm. per minute through the 2000 sieve-plates, each having pores $2\ \mu$ in diameter and $10\ \mu$ in length, which he finds in 50 cm. of stem.

It is hard to harmonize this calculation with one which has been made in this work. It has long been known that cucurbit stems when cut will exude measurable quantities of a clear watery sap from the phloem tissues. The writer watched this exudation by means of a dissecting binocular microscope giving a magnification of 150 times and it is apparently confined to the phloem with the exception of a small amount arising from the chlorenchyma sheath which surrounds the stem near the outside. The xylem sap was under tension at the time that these observations were made so that the phloem exudate was drawn into the vessels until they became blocked by the jelly which is formed upon contact of this exudate with the air.

In this experiment collections were made of the sap from 20 cut stems, the sap being removed to small test tubes by means of a pipette from the larger stems and by shaking directly into the tubes in the case of the smaller ones. Collections were made over periods of one and two minutes, the tubes corked and taken to the laboratory where the corks were removed and the weights determined, by difference, on an analytical balance. The tubes were then placed in an 80° oven equipped with a fan and thoroughly dried and reweighed to obtain dry weights. Immediately following collection portions of each stem were sectioned, projected on paper and the areas of phloem obtained at a given magnification. By higher magnification of phloem groups, areas of sieve-tubes were obtained. Individual sieve-plates were outlined, the pores were counted by means of camera lucida drawings and their areas measured from photomicrographs (plate II).

Knowing the volume delivered through a measured cross sectional area in a given time it is possible to calculate by Poiseuille's formula the pressure gradient required. The calculations will be found in a later section; it suffices here to give the value obtained, 20 atmospheres per meter in order to emphasize the lack of agreement with EWART's figures. There is serious divergence between these two sets of data. If the sieve-tube elements in EWART's stems were around 0.25 mm. in length as they were in these then there should be 2000 sieve-plates *per sieve-tube* in 50 cm. of stem. On the other hand, if his rate was 5 mm. per minute through the total phloem (it was almost double that in this case) it would have been about 150 times that or 75 cm. per minute *through the pores*. If any of the rates calculated by BIRCH-HIRSCHFELD, DIXON, MASON, or MÜNCH are multiplied by a factor which will convert them to rates through the sieve-pores and the pressures required to produce those rates calculated, the objections raised to this type of conduction will be obvious.

Granting that these criticisms are valid there remains to be found a mechanism capable of transporting the quantities of material which must move in order to provide for storage, growth, and other life processes.

The proposed mechanism

The fact that carbohydrates are synthesized in the leaves of the higher plant and that utilization of these materials takes place throughout the plant at points more or less remote from that of synthesis suggests that a maintained concentration gradient of osmotically active solutes may exist between the points of synthesis and utilization. The exudation system proposed by PFEFFER and diagramed by BLACKMAN (5) (fig. 1) also

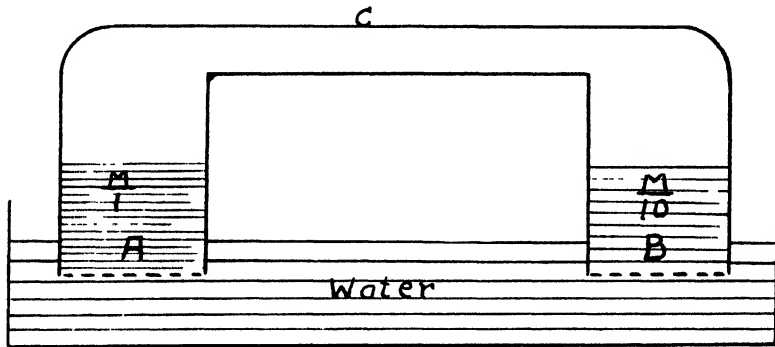


FIG. 1. Exudation system, after PFEFFER and BLACKMAN.

depends upon a maintained concentration gradient, and the continuous dilution and transport of solute in this system indicated the possibility that such a mechanism may take part in the transport of sugars within the plant.

As BLACKMAN explains this mechanism, the arm A is filled with a concentrated sugar solution, arm B contains a less concentrated sugar solution, and the connecting tube C is occupied by water. A and B are closed at their lower ends by semipermeable membranes which are bathed on the outside by pure water. Intake of water at A develops a hydrostatic pressure throughout the closed system which is greater than the maximum osmotic pressure of the solution in arm B and consequently a flow of water into A, along the tube C and out of B, takes place, carrying with it sugar from A and accumulating it at B. To maintain flow through this system the concentration at A must be maintained higher than that at B by supplying sugar to A and removing it from B. The essential point for the present purpose is that this arrangement provides a mechanism for movement of solute by osmotic action from end to end of an open conduit requiring semipermeable membranes only at the two ends.

utilization in the cambium an exudation into the xylem takes place and root pressure is produced.

There can be no doubt that the osmotic system proposed by these men would function in the translocation of solutes in the plant if all of the necessary conditions exist. The most obvious criticism of MÜNCH's proposition is the objection to conduction through the sieve-tube, which was raised in the previous section. The limitation of organic transport to plasmodesma and sieve-pores would lower the rates to values incompatible with those calculated as necessary by DIXON and others.

The mechanism proposed in the present paper is similar to that of MÜNCH except that in place of the sieve-tube with its lining of protoplasm, the total phloem, limited by the cambium or similar semi-permeable layers, is suggested as the channel for longitudinal movement.

In the diagram (fig. 3) L_1 , L_2 and L_3 are leaf cells. X represents the

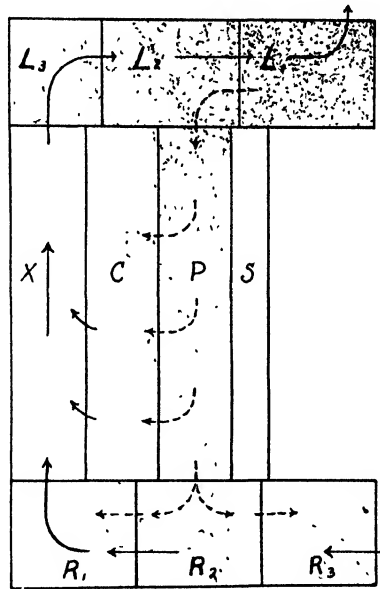


FIG. 3. System proposed to account for translocation of sugar in the phloem.

water conducting elements of the xylem, C the cambium, P the phloem, S the external limiting layer and R_1 , R_2 and R_3 root cells. The solid arrows represent the moving transpiration water, the broken ones the flow of sugar solution. The amount of stippling indicates the concentration of sugar at different levels.

The way in which sugar is delivered into the phloem is not being considered in this paper, but certain facts concerning this process cannot be disputed. Of the sugar synthesized in the leaf a major portion passes to

the vascular strands and is eventually transported to remote parts of the plant. MÜNCH has pointed out that because of the volume relationships of the leaf a movement of only $2\ \mu$ to $3\ \mu$ per hour is required to account for the passage of sugar from leaf cells to vascular tissue while much higher rates occur in the more constricted petiole and stem. Since the intercellular spaces in the leaf do not become injected with solution, or filled with crystalline solids, it seems reasonable to assume that passage takes place from cell to cell and that sugar solution is delivered to the phloem under pressure. Here, because of absorption by living cells of the phloem, cortex, and cambium and a certain loss along the rays into the wood parenchyma, the concentration is gradually diminished. Water remaining after utilization or storage of the carbohydrates and vacuolation of newly formed cells is forced into the xylem and a mass flow in the direction of lowering concentration results. A dynamic equilibrium is set up across the cambium, the relative pressure in the phloem as compared with the xylem being determined by the concentration of solutes, and the absolute pressure by the availability of water.

In examining the plant for evidence of such a mechanism, the presence of exudation pressure in the phloem is quite significant. When the stem of an active plant is dissected sap will be seen to flow from the phloem, while a subatmospheric pressure is found in the xylem if transpiration is taking place. The chlorenchyma may also exhibit a slight exudation but the pith and cortex have no tendency to lose sap. Intercellular spaces in these latter tissues are air-filled and quite prominent; but in the phloem they are lacking, or if present they are filled with solution. The cambium or a layer of small densely filled cells separates phloem and xylem, and across this layer a pressure gradient of several atmospheres often exists. On its outer side the phloem is limited by a starch sheath, endodermis, cork cambium or a layer of disintegrating phloem having air-filled intercellular spaces. These layers by virtue of active protoplasts, and either differences in degree of condensation of cell wall material, or limited available wall area, are able to confine the phloem sap and prevent its loss under pressure.

The pressure gradient between the phloem and xylem may be explained on the basis of known concentrations in these two tissues, the cambium acting as a layer of pure protoplasm exhibiting its characteristic property of semipermeability. KRAUS (41) found the sap exuded from the phloem of cucurbit fruits to contain an average of 8.7 per cent. of solids (as calculated from 17 determinations) of which as high as two-thirds might be sugar. In the present work one set of twenty-one collections from cucurbit stems gave an average dry weight percentage of 8.9, another set of twenty collections taken later in the season averaged 9.8 per cent. dry weight. MÜNCH found the concentration of water soluble materials in the sap

exuded from the bark of several species of trees to range from 14 to 31 per cent. Concentrations of xylem sap as determined by DIXON and ATKINS (19) and others are always much lower than this at the time when assimilation and downward translocation are occurring. On the other hand, DIXON'S (18) osmotic pressure determinations on leaves indicate concentrations as high as one molal. Thirty-one determinations gave an average value of 14.5 atmospheres or roughly two-thirds molal in terms of sugar. RUHLAND (57) found a concentration in palisade cells isotonic with 0.8 molal sucrose. Data collected in this laboratory on the sap expressed from pear leaves indicate concentrations approaching one molal of undissociated solute. Hexose solutions of this range of concentrations would contain from 10 to 15 per cent. solute and each gram of sugar would be associated with from 6 to 10 cc. of water occupying corresponding volumes in the leaf. When this amount of sugar has been condensed to starch or cellulose it occupies about 0.63 cc. the associated water having been almost entirely lost. It seems reasonable to assume, therefore, that the mechanism diagramed in figure 3 actually occurs in the plant, longitudinal movement taking place through the phloem.

Considering now the flow of solution within this tissue there appear to be four possible channels through which the substances may pass. These are (1) the lumina of the sieve-tubes, (2) the parenchyma cells, lumina and end walls included, (3) the walls of part or all of the phloem cells, and (4) intercellular spaces. There is no reason for assuming that all of the movement need be confined to any of these channels since the pressure gradient responsible for movement may exist throughout the space confined by the semipermeable limiting layers. The comparative importance of the different channels, therefore, depends primarily upon their relative resistance to mass flow of sugar solution and upon this basis it seems reasonable to conclude that the first two mentioned channels take little part in actual transport. In addition to the objections raised in the previous section, the movement of sugar into and out of the living cell with sufficient rapidity is a serious problem. Plasmolysis (57) and absorption (35, 36) experiments indicate that absorption of sugar by living cells is a slow process. Loss of sugar from healthy cells seems to be even more difficult to explain (36, 64). In the photosynthesizing cell we have an exception to this rule since a source of sugar is present within the protoplasm but RUHLAND'S results and those obtained in this work indicate that phloem cells are not an exception.

Intercellular spaces were few in the plants studied but where present they constitute open channels for passage. Old sieve-tubes which have lost their protoplasmic linings may also conduct but they are seldom found in active phloem. There remains the possibility that mass flow may take

place along the phloem walls. Evidence suggesting that this is the case will be presented in the following section.

Experimental results and calculations on the rates of movement of materials in the phloem

Evidence in support of the proposed type of mechanism for translocation of organic nutrients in the plant should include data on the nature of the phloem walls and a determination of their capacity to conduct. The most obvious objection to this mechanism is that phloem walls are too thin, occupying too small a proportion of the total volume. This impression may be the result of studying dehydrated mounted sections of phloem (plate IIIb). The drawings of FISHER (25) and HILL (33, 34) and the illustrations used in this work (plates IIIa, IVb and Va) show that phloem walls are relatively thick in their natural condition. Their loss of volume upon drying (plates IVa and Vb) indicates a high water content.

The readiness with which molecules of sugars, electrolytes and certain vital stains diffuse through cellulose walls demonstrates their high permeability as compared with that of the protoplasmic membranes which they enclose. Experiments by RUHLAND (57) and others indicate that phloem walls are no exception to this rule. PRIESTLEY (56) finds phloem walls to be of "amyloid" nature and free of fats. He emphasizes the relation of cell walls to the conduction of nutrients in the nutrition of the growing point. Results of the present work give additional information on this subject.

The stolon of a potato weighing 210.3 grams was sectioned, the sections mounted in tap water (plate IIIa) and ten fields, chosen at random throughout the phloem, were drawn by means of the camera lucida. Similar sections were stained in safranin in 50 per cent. alcohol, dehydrated, mounted in balsam (plate IIIb) and drawn in the same way. Other sections (plate IVa) were treated on a slide with 95 per cent. alcohol to kill them, a cover glass clamped over them to keep them flat, and dried first in a 50° oven and then in an 80° oven. The drawings from these three sets of slides were cut out and weighed and the wall area calculated as percentage of the total area. Representative fields are shown in plates IIIa, IIIb, and IVa.

These figures showing the shrinkage in only two dimensions indicate a water content constituting at least 50 per cent. of the wall volume. An observed longitudinal shrinkage of 15 per cent. and the possibility that an open structure with air spaces exists in the dry walls allow for an even greater water content.

In order to study the rate of movement of organic materials through this stolon and the magnitude of pressure gradients, projections were made of transverse sections at a magnification of 200x and the areas calculated.

TABLE I

WALLS OF THE PHLOEM OF POTATO STOLON EXPRESSED AS PERCENTAGE OF TOTAL CROSS-SECTION OF THE PHLOEM

FIELD	FRESH	DEHYDRATED IN ALCOHOL	AIR DRIED
	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>
1	40.5	25.7	16.6
2	37.0	24.3	18.6
3	36.5	25.8	18.4
4	37.3	21.7	18.9
5	36.4	30.5	17.7
6	33.9	28.0	18.6
7	39.1	26.5	18.8
8	36.6	23.1	20.8
9	37.1	23.8	16.4
10	33.3	28.9	19.9
Average	36.8 \pm 0.44	25.8 \pm 0.55	18.5 \pm 0.32

If the data given by C. F. CLARK (United States Department of Agriculture Bulletin 958) on the rate of development of tubers are plotted, a daily increment of fresh weight of 5 grams per day will be found during the period of optimum growth. The data given by C. H. JONES and B. O. WHITE (The Report of the Chemists in the 13th Annual Report of the Vermont Experiment Station, 1899-1900) indicate that mature potatoes have a dry weight of 18 to 20 per cent. The 210.3 gram tuber in this experiment had a dry weight of 16.7 per cent. Multiplying the 5-gram increment by 0.20 we find a 1-gram daily increment in dry weight and if we accept JONES and WHITE's analyses approximately 80 per cent. of this

TABLE II

AREAS OF MEASURED POTATO STOLON

	<i>mm.</i> ²	<i>mm.</i> ²
Cortex	2.49	
External phloem	1.16	1.16
Xylem	2.74	
Internal phloem	0.27	0.27
Pith	0.39	
Total	7.05	1.43 sq. mm.

is carbohydrate giving a daily increment of 0.8 gram of carbohydrate. Expressing this as a 10 per cent. solution gives us 7.2 cc. per day through 1.43 sq. mm. or $7.2 \div 0.0143 = 503.5$ cm. per day linear rate of flow, or $503.5 \div 24 = 21$ cm. per hour through the phloem, and $21 \div 0.368 = 57$ cm. per hour through the walls. This rate of flow through the walls is about the same as that calculated by DIXON for flow through the total phloem. Since no information is given as to how he treated the section of stolon which he measured, the lack of agreement cannot be explained. Rapid dehydration of sections often leads to a disproportionate shrinkage of the phloem area compared with the remainder of the section. Substituting this value in Poiseuille's formula as given by STAMM (63) we obtain a value for the pressure gradient required to deliver the solution through the walls at the rate required.

$$P = 8 \frac{Rnl}{\pi r^4}.$$

P = pressure in dynes per cm.²

R = rate of flow in cc. per sec.

n = viscosity of a 10 per cent. solution of sucrose.

l = length of gradient or tube in cm.

r = radius of conducting element in cm.

To use linear rate R_1 we substitute for R , $R_1 \times \text{area } A$; $P = 8 \frac{R_1 n A l}{\pi r^4}$; the

formula becoming $P = 8 \frac{R_1 n l}{r^2}$. R_1 in this case $= 57 \div 3600 = 0.0158$ cm. per

sec.; $n = 0.015$; $l = 1$.

$\pi r^2 = 1.43 \times 10^{-2} \times 0.368 = 0.00527$ cm.²

$r^2 = 0.00168$ cm.²

$P = 8 \times \frac{0.0158 \times 0.015}{0.00168} \times 1 = 1.13$ dynes per cm., 0.00115 g. per cm. or

1.1×10^{-6} atmospheres per centimeter.

This is the pressure gradient required to deliver the solution through the total wall area if it acted as a single pore. This value would be four times as great or 4.5×10^{-6} atmospheres per centimeter if only the space occupied by water of hydration were available. There might reasonably be expected a pressure fall of 4 atmospheres in the average stolon 5 cm. in length. This would be 0.8 atmosphere per centimeter and $0.8 \div 1.1 \times 10^{-6} = 7.15 \times 10^5$ the factor representing the possible difference in resistance between the walls acting as a single open pore and acting as they exist in the plant.

The surface presented by the cellulose molecules and the included cells is an unknown quantity in this calculation and the resistance offered can only be roughly estimated as by the calculation just presented.

In the next experiment twenty collections of sap were made from as many cucurbit stems, as previously described (page 6). The results are

TABLE III

WEIGHT OF SAP, PHLOEM AREA AND RATE OF LONGITUDINAL SAP FLOW IN CUCURBIT STEMS

STEM	ENDS	TIME	SAP		DRY AS PERCENTAGE OF FRESH WT.	PHLOEM AREA	SAP PER END PER MINUTE	LINEAR RATE OF FLOW PER MINUTE
			FRESH WT.	DRY WT.				
<i>no.</i>	<i>no.</i>	<i>min.</i>	<i>gm.</i>	<i>gm.</i>	<i>per cent.</i>	<i>cm.²</i>	<i>cc.</i>	<i>cm.</i>
1	1	2	0.0443	0.0041	9.2	0.144	0.0221	0.15
2	1	2	0.0592	0.0035	5.9	0.133	0.0296	0.22
3	1	1	0.1040	0.0111	10.7	0.064	0.1040	1.62
4	1	1	0.0409	0.0039	9.5	0.064	0.0409	0.64
5	2	1	0.1360	0.0118	8.7	0.100	0.0680	0.68
6	2	1	0.1328	0.0149	11.2	0.087	0.0664	0.76
7	1	1	0.1478	0.0139	9.4	0.181	0.1478	0.82
8	2	1	0.1517	0.0172	11.3	0.088	0.0759	0.86
9	1	1	0.0414	0.0046	11.1	0.075	0.0414	0.55
10	1	1	0.0584	0.0053	9.1	0.033	0.1168	3.54
11	1	1	0.0182	0.0021	11.5	0.017	0.0182	1.07
12	1	1	0.0317	0.0036	11.3	0.024	0.0317	1.32
13	1	1	0.0313	0.0041	13.1	0.029	0.0313	1.08
14	1	1	0.0264	0.0027	10.2	0.015	0.0264	1.76
15	1	1	0.0353	0.0029	8.2	0.041	0.0353	0.86
16	1	1	0.0838	0.0080	9.5	0.105	0.0838	0.80
17	1	2	0.1408	0.0131	9.3	0.077	0.0704	0.91
18	1	2	0.1062	0.0110	10.4	0.092	0.0531	0.57
19	1	2	0.1397	0.0131	9.4	0.058	0.0699	1.20
20	1	2	0.0916	0.0082	8.9	0.028	0.0458	1.64
			1.6215	0.1591		1.455	1.1788	

given in table III. Stems no. 1 and 2 were from an old plant starting to dry up. No. 10 was the tip of a young rapidly growing stem.

If the number of cubic centimeters, from 20 ends for one minute, be divided by the total area of the phloem in the 20 ends, we get the average

TABLE IV

PHLOEM WALL AREAS IN CUCURBIT STEMS

SECTION	CONDITION	WALL AREA PERCENTAGE OF TOTAL	PERCENTAGE OF ORIGINAL
A	Fresh	28.5	13.2
A	Dry	14.6	
B	Fresh	29.8	
C	"	27.5	
D	"	30.6	
E	Dry	14.4	

length of phloem which would have to have its volume displaced each minute in order to provide the volume collected. That is, $1.1788 \div 1.455 = 0.81$ cm. linear rate of flow. Fresh sections mounted in tap water, and air dried sections were used to estimate wall areas by camera lucida drawings.

Section A (plate Va) was mounted fresh and a field drawn, then air dried (plate Vb) and the identical field redrawn. These fields were all drawn from large bundles. In the small bundles the sieve-tubes are smaller in diameter and the walls would occupy even a larger percentage of total area. For purposes of calculation we will assume that the walls occupy 30 per cent. of the total area when wet and 15 per cent. when dry.

Sixteen average sieve-plates were drawn with the camera lucida so that the exact number of pores might be determined.

TABLE V

SIEVE-PLATE AREAS AND AREA PER PORE VALUES IN CUCURBIT STEMS

SIEVE-PLATE	NUMBER OF PORES	AREA OF SIEVE-PLATE	AREA
			NUMBER OF PORES
		$\text{cm.}^2 \times 10^{-5}$	$\text{cm.}^2 \times 10^{-6}$
1	37	1.965	0.531
2	42	2.263	0.540
3	45	1.965	0.437
4	46	1.208	0.263
5	50	1.058	0.211
6	56	3.465	0.620
7	62	2.420	0.391
8	63	3.020	0.480
9	69	2.567	0.373
10	71	3.020	0.426
11	71	3.780	0.532
12	75	3.175	0.424
13	76	3.020	0.397
14	97	3.175	0.327
15	108	3.175	0.294
16	113	3.465	0.267
Total	1,081	42.741×10^{-5}	

Dividing 42.74×10^{-5} by 1081 we find that each pore is allotted an averaged area of $0.395 \times 10^{-6} \text{ cm.}^2$ or $39.5 \mu^2$.

The pores in these plates were from 1.25μ to 1.75μ in diameter and the plates varied from 3μ to 10μ in thickness with an average of about 5μ .

A number of phloem groups were projected at a magnification of 200 times. These sections were all mounted in tap water, a few having been

stained in dilute acid violet solution first. The figures in table VI give an idea as to the percentage of phloem area occupied by sieve-tubes.

TABLE VI
PERCENTAGE OF PHLOEM OCCUPIED BY SIEVE-TUBES IN CUCURBIT STEMS

STEM	OUTER GROUP		INNER GROUP	
	NUMBER OF TUBES	AREA PERCENTAGE OF TOTAL	NUMBER OF TUBES	AREA PERCENTAGE OF TOTAL
1	131	21.2	110	23.6
2	88	18.1	80	18.1
3	54	10.7	70	19.4
4	53	18.9	52	21.6
5	41	14.3	36	14.8
6	79	13.4	101	20.7
7	85	20.8	122	27.9
8	140	16.8	121	19.7
9	73	14.4	73	18.1
10	98	18.9	57	23.0
11	51	19.1	40	22.6
12	33	13.2	44	13.2
13	55	15.3	52	18.1
14	34	15.5	48	19.1
15	42	15.0	40	16.6
16	74	19.5	84	23.0
17	109	16.2	93	15.6
18	30	17.1	42	18.1
19	66	13.4	73	12.0
20	72	14.1	74	13.1

If we may assume that the sieve-tubes occupy 20 per cent. (45) of the total phloem area we have the data from which to calculate the pressure gradient necessary to deliver through the sieve-tubes and through the walls, the volume of sap collected. Using EWART'S figure, 2μ , for the diameter of pores we find the area of the pore to be $3.14\mu^2$, and this represents $3.14 \div 39.5 \times 100$ or 7.95 per cent. of the area of the sieve-plate.

The linear rate of flow through these pores = $0.81 \div 0.20 \div 0.0795 = 51$ cm. per min. or 0.85 cm. per sec. = R_1 .

$$n = 0.015$$

$$l = 5 \times 10^{-4} \text{ cm.}$$

$$r = 1 \times 10^{-4} \text{ cm.; } r^2 = 1 \times 10^{-8} \text{ cm.}^2$$

$$p = \frac{8 \times 0.85 \times 0.015 \times 5 \times 10^{-4}}{1 \times 10^{-8}} = 5100 \text{ dynes,}$$

5.2 g. or 0.005 atmosphere pressure gradient to maintain flow through one

set of sieve-plates. A measure of 74 sieve-tube elements in these stems gives a value 0.26 mm. average length, that is, about 4 sieve-plates per mm., 40 per cm., or 4000 per meter. Thus it would necessitate a pressure gradient of 20 atmospheres per meter to maintain this rate of flow in these stems. If all of the dry weight of the sap were sucrose a maximum osmotic pressure of but 8 atmospheres could be developed, which would be inadequate to cause the observed exudation. In view of the rates of from two to four times the above value which have been observed, and also of the fact that the pores seem to be filled with protoplasm rather than open, or at least obstructed by a cellulose lamella, it would seem impossible to explain the exudation on the basis of mass flow through the sieve-tubes.

Examining now the possibility for movement through the walls the linear rate per cm. would be:

$$\begin{aligned} R_1 &= 0.81 \div 60 = 0.0135 \text{ cm. per sec. through the phloem} \\ &= 0.045 \text{ cm. per sec. through the phloem walls} \\ n &= 0.015; l = 1 \text{ cm.} \end{aligned}$$

$\pi r^2 = 1.455 \div (20 \times 20) \times 0.3 = 0.00109 \text{ cm.}^2$ for walls per phloem group and $r^2 = 0.000348$ considering the walls of each phloem group as if they formed a single capillary tube. Then $p = \frac{8 \times 0.045 \times 0.015}{0.000348} = 15.5$ dynes per cm. or 1.5×10^{-5} atmosphere per cm. gradient through the walls of 20 phloem groups, there being 10 bicollateral bundles in the internode. If the same calculation is made using 15 per cent. of the total area as occupied by the free space filled with solution in the walls the value 6.1×10^{-5} atmosphere per cm. is obtained. It seems probable, therefore, that the greater portion of the exuded sap from cucurbit stems flows out through the walls rather than through the sieve-tubes.

Another series of collections was made over longer time intervals and the following data collected.

Dividing the total volume 1.2589 cc. by 0.0918 we find that a volume of solution equal to the volume of the total phloem in 13.7 cm. of stem flowed out during the 21 minutes, about 1 cm. of stem having been removed during the collection.

These data bring out two interesting points in this work. The proteinaceous material in the cucurbit sap gelatinizes rapidly upon exposure to air and tends to clog the ends of the cut stems. The removal of a thin section allows flow to be resumed at about its former rate. This rate, however, drops off rapidly at first, and then more slowly, approaching asymptotically a value of about 0.3 cm. per minute.

The peduncle of a mature pumpkin was sectioned as was the stem below and above the fruit.

TABLE VII
SAP FLOW FROM CUCURBIT STEMS

COLLECTION NUMBER	FRESH WEIGHT OF SAP	DRY WEIGHT OF SAP	DRY WEIGHT PERCENT- AGE OF FRESH	PHLOEM AREA	LINEAR RATE OF FLOW	TIME	RATE $\frac{\text{CM.}}{\text{MIN.}}$
Collection A			Pumpkin stem				
				cm.^2	cm.	min.	
1	0.1190	0.0101	8.5	0.173	0.688	1	0.688
2	0.0660	0.0046	7.0		0.381	1	0.381
3	0.0800	0.0056	7.0		0.463	2	0.231
4	0.0465	0.0031	6.7		0.269	4	0.067
Collection B	Large pumpkin stem. Collection made for 5 minutes from both ends						
	0.9484	0.0878	9.2	0.175	5.43	5	0.543
Collection C			Peduncle of a growing pumpkin				
1	0.1683	0.0147	8.7	0.0918	1.833	1	1.833
2	0.0593	0.0041	6.9		0.646	1	0.646
3	0.1005	0.0074	7.4	*	1.094	1	1.094
4	0.1028	0.0081	7.9	*	1.120	1	1.120
5	0.0916	0.0070	7.6		0.997	1	0.997
6	0.0575	0.0042	7.3		0.627	1	0.627
7	0.1404	0.0103	7.3	*	1.530	2	0.765
8	0.1971	0.0158	8.0		2.240	4	0.560
9	0.2074	0.0165	8.0	*	2.800	5	0.560
10	0.1340	0.0099	7.4	*	1.460	4	0.365

* This stalk was cut 5 times during the collection, thin sections about 1-2 mm. in thickness being sliced off with a safety razor blade. Collection made from stem end.

The pumpkin weighed 18,940 gm. of which 9 per cent. was dry weight. If this fruit were made from a 9 per cent. solution in 100 days then 189.4 cc. flowed in per day, 7.9 cc. per hour or 0.13 cc. per minute. This divided by 0.450 gives a linear rate of 0.292 cm. per min. The smaller size of the

TABLE VIII
AREAS OF PEDUNCLE AND STEM OF PUMPKIN

TISSUE	PEDUNCLE	STEM ABOVE	STEM BELOW
	cm.^2	cm.^2	cm.^2
Phloem	0.450	0.084	0.240
Xylem	0.197	0.042	0.154
Ground tissue	3.160	0.800	1.017

developing peduncle and the difference in gradient and viscosity between day and night and bright and dull days would necessitate a somewhat greater rate than this during part of the time at least. The general agreement in this figure indicates, however, that rates of from 0.3 to 0.5 cm. per minute through the phloem of these stems may be expected. Since the pressure gradient necessary to produce rates through the sieve-tubes proportional to these rates through the phloem cannot be accounted for by the concentrations found in these elements it seems much more likely that, at least in the case of cut stems, the observed volumes of solution moved out through the walls. The gradient necessary to produce a linear rate of movement of 0.3 cm. per minute would be around 1.14×10^{-5} atmosphere per cm. for movement through the phloem walls acting as a single capillary. An actual gradient of about 0.05 atmosphere per cm. might be expected, giving a factor of 4400 to represent the difference in resistance between the phloem walls, acting as a single capillary, and acting as they do in the plant.

MÜNCH (48) has given a value for the linear rate of flow necessary to provide for growth in several forest trees. Assuming movement to take place through the sieve-tubes, he calculated a rate of from 16 to 31 cm. per hour for an average 120 day growth period. The walls may well occupy as much or more space than the sieve-tubes in these stems, so comparable rates would probably occur if they were the channels. Rates of from 2.81 to 4.46 per hour occurred in the stems of fruits.

Calculations based on data collected in this laboratory on the rate of increase of dry weight in the pear tree give comparable rates. A group of 16 two-year-old trees of uniform size and shape were used. They were taken out two at a time and analyzed. The following data have been selected and tabulated (table IX). The last four columns give values for the osmotic pressure at 0° C., calculated from the freezing point lowering of expressed sap of the bark.

The average diameter at the top of the trunk for these trees was 3.10 cm. Measurements were made of the thickness of phloem and cortex of seven trunks of this diameter. Strips of bark were taken from the north and south sides of the trunks and microtome sections of these were projected at a magnification of 100x, outlined and measured. Table X gives the data on these. In table XI the cell wall: phloem area ratios obtained from camera-lucida drawings of these sections are given. The average percentage of the phloem area occupied by walls in the fresh sections was 40.54 per cent. and in the air dried sections 23.50 per cent. The average thickness of cortex was $\frac{275.7}{190}$ or 1.45 mm. and of phloem $\frac{84.89}{255}$ or 0.33

TABLE IX
COMPOSITION OF PEAR TREES REMOVED AT INTERVALS THROUGHOUT THE YEAR

TREE	DATE REMOVED	TRUNK AND ROOTS			SAP CONCENTRATION OSMOTIC PRESSURE AT 0° C.			
		DRY WEIGHT	STARCH	DIAMETER AT TOP OF TRUNK	LEAVES	BRANCHES (BARK)	TRUNK (BARK)	ROOTS
<i>no.</i>		<i>gm.</i>	<i>per cent.</i>	<i>cm.</i>	<i>atm.</i>	<i>atm.</i>	<i>atm.</i>	<i>atm.</i>
1393	2/13	854.4	8.19	2.50	—	17.81	20.45	12.40
1383	2/15	756.6	7.01	2.88	—	20.42	20.58	13.72
1398	3/14	881.1	8.22	2.69	—	16.65	17.70	12.68
1377	3/18	729.8	7.47	2.57	—	15.19	18.05	12.60
1354	4/22	800.4	6.04	2.52	11.68	11.55	12.64	11.59
1330	4/24	618.4	2.91	2.90	13.97	9.82	10.71	10.59
1352	6/9	751.0	3.18	3.00	16.18	10.92	9.27	9.90
1327	6/12	755.2	5.84	2.92	23.82	15.28	9.75	9.66
1334	7/30	772.3	10.36	3.25	22.42	12.08	9.14	10.71
1358	8/4	862.7	9.98	3.15	24.21	12.42	10.48	11.28
1309	10/13	1187.3	12.41	2.45	22.20	14.17	13.42	11.71
1284	10/20	1439.9	12.12	3.30	28.07	17.64	15.76	12.10
1305	12/17	1446.7	9.76	3.68	48.50	19.64	17.88	14.08
1331	12/18	1990.3	12.86	4.20	—	20.80	18.96	13.81
1280	2/24	1749.5	7.85	3.92	—	18.59	20.21	13.35
1255	2/26	1792.5	9.22	3.70	—	19.56	18.78	13.12

mm. Denoting the total radius as r , the cortex thickness as c , and the phloem thickness as p , the area of the phloem would be

$$\pi (r - c)^2 - \pi [r - (c + p)]^2; r = 1.55 \text{ cm.}$$

$c = 0.145 \text{ cm.}$, $p = 0.033 \text{ cm.}$ and the phloem area is 0.30 cm.^2

From the data of MÜNCH (48) and CHANDLER (10) and the osmotic concentrations recorded in table IX it seems reasonable to assume a concentration of about two-thirds molal in these tissues. CHANDLER (10) finds a theoretical molecular weight of about 200 for these solutes and the vertical gradients of sucrose and reducing sugars found by MASON and MASKELL (47) indicate that the phloem sap should at least not fall below this average.

A two-thirds molal solution of a substance of molecular weight 200 would have a percentage composition of 11.77 per cent. solute. Table IX shows that between August 1 and December 18 there was a daily increase in dry weight of 6.53 grams per tree which at the above concentration would correspond to a volume of 55.6 cc. flowing daily or 2.32 cc. per hour. This volume through 0.30 cm.^2 would require a linear rate of 7.73 cm. per hour through the total phloem or about 19 cm. per hour through the walls and would necessitate a pressure gradient of 1.60×10^{-8} atmosphere per centi-

TABLE X
THICKNESS OF PHLOEM AND CORTEX OF PEAR BARK

SAMPLE NO.	PHLOEM			CORTEX		
	NUMBER OF MEASUREMENTS	TOTAL THICKNESS	AVERAGE THICKNESS	NUMBER OF MEASUREMENTS	TOTAL THICKNESS	AVERAGE THICKNESS
		<i>mm.</i>	<i>mm.</i>		<i>mm.</i>	<i>mm.</i>
1N	25	6.57	0.263	13	18.8	1.45
1S	20	4.37	0.218	7	10.9	1.56
2N	22	6.30	0.286	14	13.9	0.99
2S	25	9.76	0.391	15	14.3	0.94
3N	15	7.37	0.491	16	22.1	1.38
3S	13	6.20	0.477	12	20.2	1.68
4N	17	6.13	0.361	14	20.0	1.43
4S	20	6.17	0.308	15	22.1	1.47
5N	16	4.72	0.295	16	22.3	1.39
5S	16	5.18	0.323	15	20.0	1.33
6N	18	5.83	0.324	14	24.0	1.72
6S	17	6.69	0.393	13	22.7	1.75
7N	17	4.80	0.282	14	23.4	1.67
7S	14	4.80	0.343	12	21.0	1.75
Total	255	84.89		190	275.7	

TABLE XI
CELL WALL : PHLOEM AREA RATIOS OF PEAR BARK

SAMPLE NO.	WEIGHT			WALLS, PER CENT. TOTAL PHLOEM
	WALLS	LUMINA	TOTAL	
	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>per cent.</i>
1 N a	2.16	2.97	5.13	42.1
b	1.51	2.21	3.72	40.6
1 S a	2.23	2.85	5.06	43.8
b	1.42	1.91	3.33	42.7
2 N a	1.79	2.96	4.75	37.7
b	2.81	4.74	7.55	37.2
2 S a	1.85	2.70	4.55	40.7
b	2.18	3.06	5.24	41.7
3 S a	2.97	5.14	8.11	36.6
b	2.82	4.39	7.21	39.1
4 Fresh	2.01	2.41	4.42	45.5
Air dried	1.30	3.69	4.99	26.2
5 Fresh	2.85	4.49	7.34	38.8
Air dried	1.54	5.86	7.40	20.8

meter for the total phloem walls acting as a single capillary. Assuming a gradient of 0.05 atmosphere per centimeter possible in the tree, a factor 3.1×10^6 represents the relative resistance of the walls compared to a single capillary of equal cross sectional area.

Rates of flow through petioles have been calculated by both DIXON (21) and BIRCH-HIRSCHFELD (4). In order to check DIXON's rate, five leaves of *Tropaeolum majus* were measured and fresh sections of the petioles were projected, the phloem groups outlined, and the areas determined with the planimeter. Using the assimilation rate which DIXON quotes from BROWN

TABLE XII

AREAS OF LAMINA AND OF PETIOLE PHLOEM OF *Tropaeolum* LEAVES

LEAF	LAMINA AREA	AREA OF PHLOEM OF PETIOLE
	cm. ²	cm. ²
1	71.9	0.00476
2	70.5	0.00371
3	75.0	0.00345
4	75.5	0.00394
5	62.8	0.00359
Totals	355.7	0.01945

and MORRIS these leaves would form 0.03557 gram per hour or 0.3557 gram in 10 hours and two-thirds of this or 0.3271 gram would be transported through the petiole in 24 hours. Using the factor 10 to arrive at a volume, 2.371 cc. would pass through 0.01945 cm.² of phloem in 24 hours

at a linear rate of $\frac{2.371}{0.01945 \times 24}$ or approximately 5.1 cm. per hour. The walls were not measured in this case but 30 per cent. would be a conservative estimate, and through this area the rate would be 17.0 cm. per hour.

BIRCH-HIRSCHFELD used the leaf of *Phaseolus multiflorus* in her calculation. She assumed an hourly loss of 0.28 gram from 1 sq. meter and $\frac{0.28 \times 117}{100^2}$ or 0.003276 gram per hour from her leaf. Through the 0.004598 cm.² of phloem this would be 0.71 cm. per hour for the pure solute, or 7.1 cm. per hour for about a 10 per cent. solution. Through the 37.2 per cent. of the phloem occupied by walls this would be 19.1 cm. per hour. A *Phaseolus* leaf which was measured to check this result had a lamina of 64 cm.² and the phloem measured 0.0032 cm.² Using the same rate of loss from the leaf, a flow of 5.58 cm. per hour through the total phloem, or 15 cm. per hour through the walls, would be adequate.

SACHS (59) found a rate of assimilation of 1.8 grams per square meter per hour in *Helianthus annuus*. With this same plant BROWN and MORRIS (7) give rates around 1 gram per square meter per hour. BROWN and ESCOMBE (8), measuring CO₂ absorption, arrive at rates which are less than one-half of this, and THODAY (66) using an improved one-half leaf method got an average value of 1.65 grams per square meter per hour.

Among the later workers KOSTYTSCHEW (40) recorded rates of assimilation in several species arrived at by measuring CO₂ absorption. His values approximate the early figure of SACHS, and BOYSEN-JENSEN (6) using a similar method finds values of from 0.5 to 1.0 gram per square meter per hour. Since measurements of the phloem in the petioles are not available in any of these experiments a set of determinations was made on *Phaseolus* leaves using the stamping method of THODAY (66), weighing the changes in dry weight and measuring the phloem in the petioles from projections of sections. The center leaflets of the compound leaves were used, a four square-centimeter area being stamped on each side of the midrib.

The plants were moved from the greenhouse to a dark room at 4:00 P. M. on February 20. The leaves were stamped and tagged between 8:00 and 8:30 P. M. the same evening and the experiment started the following morning. Four sets of ten leaves each were used; eight samples were collected falling into five groups. The four sets will be called *ab*, *ac*, *cd*, and *ce*, each letter designating a sample. The *a* samples were collected at 8:00 A. M. February 21, after 16 hours in the dark room attached to the plants. The *b* sample was collected at 6:00 P. M. February 21 after 16 hours in the dark room attached to the plant, followed by 10 hours illumination detached from the plant with petioles in water. The *c* samples were collected at 6:00 P. M. February 21, after 16 hours in the dark and 10 hours illumination attached to the plant. The leaves of the *d* sample, after the same treatment as *c*, were detached and the petioles placed in water, the *d* sample being collected at 10:00 A. M. February 22, after 14 hours in the dark. The *e* sample had the same treatment except that these leaves remained attached throughout.

The printed leaf areas were cut out with scissors, placed with a drop of ether in a small test tube, brought to 100° C. in a boiling water bath, dried in a 55° oven for 24 hours and brought to constant weight in a 95° oven. The two *a* samples and the *e* sample were theoretically similar and weighed nearly alike, so their weights were averaged. The three *c* samples were all similar, so their weights were also averaged. The weights were:

Sample weight of 40 cm.²

a 0.0773 g. 16 hours in dark

b 0.0912 " " " " " + 10 hours illuminated, detached

c	0.0832 g. 16 hours in dark + 10 hours illuminated, attached
d	0.0798 " " " " " " " " " " " " + 14 hours dark detached
e	0.0773 g. 16 hours in dark + 10 hours illuminated, attached + 14 hours dark attached
	Weight differences per 40 cm. ²
b-a	0.0139 g. assimilation in 10 hours illumination, detached leaves
c-a	0.0059 " " " " " " " " attached "
c-e	0.0059 " respiration and translocation 14 hours in dark, attached leaves
c-d	0.0034 g. respiration in 14 hours in dark, detached leaves
b-e	0.0080 " translocated during 10 hours illumination, attached leaves
d-e	0.0025 g. translocated during 10 hours dark, attached leaves

The extraordinarily good agreement of these figures is accidental and resulted from the averaging indicated. The general order of magnitude, however, should be reliable. The value 0.0080 g. translocated in 10 hours from 40 square centimeters gives an hourly loss of 0.2 g. per hour per square meter. Table XIII gives the areas of these leaves and table XIV the areas of the phloem in the petioles.

TABLE XIII

AREAS OF *Phaseolus* LEAVES IN SQUARE CENTIMETERS

LEAF	ab	ac	cd	ce
	cm. ²	cm. ²	cm. ²	cm. ²
1	33.5	38.4	21.9	26.4
2	40.7	27.1	32.5	26.6
3	33.9	24.8	22.2	38.2
4	35.6	34.5	34.4	22.2
5	44.1	43.0	30.2	35.4
6	24.5	22.0	38.9	25.9
7	20.0	25.8	42.0	37.1
8	22.6	29.4	28.4	34.5
9	23.9	24.6	21.8	35.7
10	26.6	33.9	27.8	34.6
Total	305.4	303.5	300.1	316.6
Average	30.54	30.35	30.01	31.66

The average leaf area was 30.64 cm.², and the average phloem area 0.123 mm.² or 0.00123 cm.². A transport of 0.2 g. per square meter = 0.00002 g. per square centimeter per hour and $30.64 \times 0.00002 = 0.0006128$ g. or 0.006128 cc. of a 9 per cent. solution. $\frac{0.006128}{0.00123} = 5$ centimeters per hour

linear rate of flow through the entire phloem. Table XV gives the cell wall: phloem area ratios of seven of these petioles. Dividing 5 by 0.372 we arrive at a rate of 13.5 cm. per hour through the walls.

TABLE XIV

AREAS OF PHLOEM IN *Phaseolus* PETIOLES IN SQUARE MILLIMETERS

LEAF	ab		ac		cd		ce	
	PHLOEM	PETIOLE	PHLOEM	PETIOLE	PHLOEM	PETIOLE	PHLOEM	PETIOLE
	<i>mm.</i> ²	<i>mm.</i> ²	<i>mm.</i> ²	<i>mm.</i> ²	<i>mm.</i> ²	<i>mm.</i> ²	<i>mm.</i> ²	<i>mm.</i> ²
1	0.161	2.07	0.134	1.86	0.106	1.15	0.093	1.19
2	0.208	2.64	0.126	1.63	0.125	1.74	0.132	1.61
3	0.139	1.94	0.078	1.26	0.078	1.01	0.117	1.66
4	0.203	2.64	0.119	1.79	0.137	1.54	0.091	1.03
5	0.252	2.85	0.171	2.05	0.130	1.59	0.150	1.86
6	0.103	1.39	0.098	1.21	0.127	1.96	0.095	1.16
7	0.103	1.08	0.082	1.03	0.147	2.06	0.092	1.08
8	0.112	1.75	0.102	1.25	0.088	1.26	0.125	1.47
9	0.100	1.96	0.096	1.02	0.074	0.88	0.147	1.66
10	0.116	1.66	0.139	1.66	0.104	1.03	0.130	1.87
Total	1.497	18.98	1.145	14.76	1.116	14.72	1.172	14.59
Average value	0.1497		0.1145		0.1116		0.1172	

TABLE XV

CELL WALL: PHLOEM AREA RATIOS OF *Phaseolus* PETIOLES

SAMPLE NUMBER	RATIO EXPRESSED AS PER CENT.
1	41.2
2	34.4
3	36.0
4	34.9
5	34.1
6	40.8
7	38.9
Average	$260.3 \div 7 = 37.2$ per cent.

If the mechanism pictured in the previous section actually acts in the transport of organic materials through the phloem of woody stems it should be possible to force certain solutions through this tissue. Apparatus

was designed which allowed the application of pressure to solutions covering the exposed bark of stems and the rates of movement measured under certain standard conditions. After some experimentation a strong solution of Orange G mixed with Higgin's India Ink was decided upon as an indicating solution. A five-centimeter length of stem about 2 centimeters in diameter was used, a strip of bark one centimeter wide being removed from each end leaving an intact strip 3 centimeters wide in the center. The wood of the upper end was coated with hot paraffin and the piece of stem sealed into a glass tube with plasticine and cotton string. The indicator solution was poured in and pressure applied by means of a plunger in the glass tube. Several species were studied by this method. The maximum and minimum distances were measured, the average was estimated visually.

In all of these tests a standard length of 3 centimeters was used. Preliminary tests showed, however, that the rate was inversely proportional to

TABLE XVI

RATE OF MOVEMENT OF ORANGE G SOLUTION THROUGH THE BARK OF HARDY PEAR STEMS.¹
MARCH 13, 1930

PRESSURE	TIME	DISTANCE		
		MINIMUM	AVERAGE	MAXIMUM
<i>atm.</i>	<i>min.</i>	<i>cm.</i>	<i>cm.</i>	<i>cm.</i>
1	1	0.50	0.60	1.50
1	1	0.30	0.60	1.50
1	2	0.50	1.00	2.00
1	2	0.50	0.75	1.50
1	4	0.75	1.00	3.00
1	4	0.50	1.00	2.50
1	8	1.00	2.00	3.00
1	8	1.00	2.25	3.00
1	12	3.00	3.00	3.00
1	12	2.50	3.00	3.00
$\frac{1}{2}$	4	0.30	0.50	1.00
2	4	1.50	2.00	3.00
3	4	2.00	3.00	3.00
4	3	2.50	3.00	3.00
1 check ²		0.30	0.50	1.00

¹ The buds were swelling and the bark could be peeled off of these stems rather readily.

² The check in each case was treated like the other samples, except that as soon as the pressure of one atmosphere was developed the stem was removed and the measurements made. Similar treatments in which no pressure was applied showed practically no movement of the dye.

the length just as would be expected from Poiseuille's formula. The following tables give the data on these tests.

Acid dyes moved readily through these tissues but basic dyes were adsorbed by the walls and scarcely entered the bark. India ink proved effective in stopping the intercellular spaces of the cortex and confining the

TABLE XVII

MOVEMENT OF ORANGE G SOLUTION THROUGH THE BARK OF MADRONE STEMS (*Arbutus* sp.)

PRESSURE	TIME	DISTANCE		
		MINIMUM	AVERAGE	MAXIMUM
(a) Dormant stems with bark sticking. March 15, 1930				
<i>atm.</i>	<i>min.</i>	<i>cm.</i>	<i>cm.</i>	<i>cm.</i>
1	1	0.10	0.30	1.50
1	2	0.50	0.75	2.50
1 . . .	4	0.50	1.50	3.00
1	8	1.00	2.00	3.00
1	12	2.50	3.00	3.00
$\frac{1}{2}$	4	0.40	1.00	1.50
2	4	1.00	2.00	3.00
3	4	1.00	2.50	3.00
4	3	1.00	2.50	3.00
1 check		0.10	0.20	0.50
(b) Stems with bark slipping. March 17, 1930				
1	1	0.25	0.33	1.00
1 . . .	2	0.33	0.60	2.00
1 . . .	4	0.50	1.00	2.50
1	8	0.50	1.50	3.00
1	12	1.00	2.50	3.00
$\frac{1}{2}$	4	0.33	0.75	2.50
2 .	4	0.50	1.00	3.00
3	4	1.00	2.00	3.00
4	3	1.00	2.50	3.00
1 check		0.25	0.30	0.60
(c) Stems with bark slipping. Orange G + India ink. March 17, 1930				
1	1	0.20	0.30	0.70
1 . . .	2	0.20	0.40	0.70
1 . . .	4	0.33	0.75	2.00
1 . . .	8	0.50	1.33	2.50
1 . . .	12	1.00	1.50	2.50
$\frac{1}{2}$. . .	4	0.50	0.75	1.50
2 . . .	4	0.50	1.33	2.60
3 . . .	4	1.00	1.50	3.00
4	3	1.00	2.00	3.00
1 check .		0.20	0.20	0.20

TABLE XVIII

MOVEMENT OF ORANGE G SOLUTION THROUGH THE BARK OF WILLOW,
MARCH 14, 1930

PRESSURE	TIME	DISTANCE		
		MINIMUM	AVERAGE	MAXIMUM
<i>atms.</i>	<i>min.</i>	<i>cm.</i>	<i>cm.</i>	<i>cm.</i>
1	1	0.25	0.30	0.50
1	2	0.33	0.50	1.50
1	4	0.50	1.00	2.00
1	8	1.50	2.00	3.00
1	12	2.00	3.00	3.00
$\frac{1}{2}$	4	0.33	0.75	1.50
2	4	1.00	2.00	3.00
3	4	2.00	2.50	3.00
4	3	3.00	3.00	3.00
1 check		0.10	0.15	0.20

TABLE XIX

MOVEMENT OF ORANGE G SOLUTION + INDIA INK THROUGH THE BARK OF BARTLETT PEAR,
APRICOT, EUCALYPTUS, AND REDWOOD, MARCH 19, 1930

PRESSURE	TIME	DISTANCE					
		MINIMUM		AVERAGE		MAXIMUM	
(a) Bartlett pear. Bark slipping, buds swelling							
<i>atm.</i>	<i>min.</i>	<i>cm.</i>	<i>cm.</i>	<i>cm.</i>	<i>cm.</i>	<i>cm.</i>	<i>cm.</i>
1	4	*0.50	*0.50	*0.75	*0.75	*2.00	*1.50
1	8	1.00	1.00	2.00	1.50	3.00	3.00
1	12	1.00	1.00	2.00	2.00	3.00	3.00
(b) Apricot. Bark slipping. Leaves $\frac{1}{2}$ size							
1	4	1.00	1.00	1.50	1.25	2.00	2.00
1	8	1.00	1.00	2.00	2.00	3.00	2.50
1	12	2.00	1.50	2.75	2.50	3.00	3.00
(c) Eucalyptus. Bark slipping							
1	4	1.00	0.50	1.50	1.00	2.50	2.00
1	8	1.00	1.00	1.50	1.50	2.00	2.50
1	12	1.50	1.50	2.00	1.50	3.00	1.50
(d) Redwood. Bark slipping partially							
1	6	0.33	0.50	0.75	0.75	1.50	1.50
1	12	1.00	1.00	1.50	1.33	2.00	2.50
1	18	1.00	0.50	2.00	1.50	3.00	3.00

* Duplicate experiments.

movement to the phloem. Microscopic examination showed the presence of the dye in the walls of the phloem tissues and there was no tendency for it to be localized in sieve-tubes.

Discussion

Since methods are not known at the present time by which the movement of organic materials through intact plants may be studied it is extremely difficult to prove that any particular tissue system is primarily involved in this process. The ringing experiments of CURTIS, MASON and MASKELL, and others leave little doubt but that transport of organic materials actually occurs in the bark. The sieve-tubes and the parenchyma cells with streaming protoplasm, however, both seem inadequate as conducting elements, while certain data presented in this paper indicate that the cell walls of the phloem may possibly act as the channels. From 30 to 40 per cent. of the volume of the phloem is occupied by wall material composed of about equal parts of cellulose and water. The rates calculated for flow through the walls while much too great to be explained by diffusion, seem not unreasonable in view of the possible maximum pressure gradients in the phloem which may be inferred from the exudation studies on cucurbit stems. The fact that acid dyes which move readily in the phloem walls may be forced through the bark of woody stems at rates which are similar to those calculated as necessary for storage (21) and growth (48) provides additional evidence for passage through the walls.

All of this evidence, however, is indirect and much remains to be done before any of the present theories can be accepted. In the meantime any proposed mechanism must be judged upon its general feasibility, certain characteristics being indispensable. Nourishment of the meristems and provision for growth and storage necessitate fairly rapid transport (9) over considerable distances. The development of tubers and flow through the trunks of tall coniferous trees are examples which present serious difficulty. The magnitude of rates in the former case, and the lack of vessels in the xylem and of sieve-pores in the phloem in the latter, practically preclude the application of previously described theories. In fact, the assumption that translocation takes place through sieve-tubes has probably hindered an unbiased study of this problem ever since these elements were named by HARTIG. Besides the resistance due to the narrowness of the pores and the fact that actual pores have not been demonstrated in many cases, the entire lack of sieve-tubes in bundle terminals of leaves and the question as to their state of differentiation in intercalary meristems are additional objections.

In considering the mechanism proposed in this paper in its relationship to the plant there are many questions yet to be settled. The state of

hydration of the phloem walls in the intact plant and the intermolecular force with which the water is held can only be surmised. It seems reasonable that the walls should occupy at least as much volume in their natural state as they do in cut sections but no actual data on this point are available.

The presence of organic solutes within the phloem walls is difficult to determine and their concentrations may only be roughly estimated. Considering the thinness of the phloem cylinder, the rigidity of the enclosing layers and the low compression coefficient of watery solutions it may be assumed that so long as the rate of flow is not too great, a relatively high concentration will be maintained throughout the phloem, the effective gradients becoming fairly short. The large surface exposed and the short distance to the xylem favor this view. This factor should considerably reduce the rates necessary especially in remote parts of the plant.

It is not essential to the proposed mechanism that there exist between "source" and "sink" a concentration gradient in the tissues as a whole. The ability of living non-photosynthesizing cells to produce a low concentration within their walls is all that is necessary. This possibly explains the lack of evidence for such gradients in much analytical data. The vertical gradients of MASON and MASKELL (47) probably represent the vacuolar concentrations at different levels more than they do cell wall contents, and reflect accumulation within living cells rather than conditions within the conducting tracts.

In addition to the problems already mentioned there remains the crucial one of determining the absolute resistance offered by the hydrated colloidal jell, of which the walls are composed, to mass flow of solutions. Immense amounts of internal surface are undoubtedly exposed but how this surface, composed presumably of longitudinal chains of carbohydrate molecules (62) reacts in comparison with the rigid walls of a glass capillary can only be surmised. The structure is less rigid than that of lignified walls, and water seems to be held less firmly than by agar or gelatin jells. The fact remains, however, that the spaces through which it is proposed that food materials in solution are passing are of molecular dimensions and irregular in form. Since no formulae are available by which to calculate pressure gradients and rates of flow through this type of material mathematical treatment is impossible. Use of Poiseuille's formula leads to high values for the required gradients and indicates discrepancies fully as great as the ones which appeared in the sieve-tube calculations. It remains for future experiments to settle this phase of the problem.

In the face of these obvious weaknesses in the proposed theory one might seem overoptimistic in attempting to view it in relation to the plant;

yet when this is done several interesting deductions become manifest. Considering the nutrition of the growing points, the exudation pressures which have been demonstrated in the phloem should force nutrients in solution into the meristems of root and shoots (51, 52) more effectively than root pressure (50, 53) or the osmotic system recently described by PRIESTLEY (56). In secondary roots the initiation of a cork cambium (54) within the phloem would be a natural result of facilitated nutrition. In storage roots, where the cork cambium surrounds a fleshy cortex, nutrition is effected through a disrupted endodermis. In primary roots an intact endodermis results in an early breakdown in the cortex. Radial arrangement in the region behind the growing root-tip allows water absorption to take place independently of the movement of foods.

The subdivision of the phloem observed to occur in storage organs such as the potato tuber, while narrowing the individual elements of this tissue, results in an increase in volume as well as in the ratio of cell wall to total phloem volume. Contrary to previous opinion (1, 34) this should increase its ability to conduct.

The fact that living cells (14) are essential to the functioning of the mechanism proposed in this work cannot be questioned. The semipermeability of the membranes separating the phloem from the adjacent tissues is directly dependent upon the nature of living protoplasm. Any condition tending to increase this permeability will allow excess leakage, and translocation will be hindered. The injection of osmotically active solutes into the phloem is dependent upon the synthetic activity of chlorophyll-bearing cells or the hydrolytic activity of storage elements. Finally the withdrawal of these substances from the channels of movement depends upon the ability of living cells to accumulate, condense, or oxidize these substances in their normal life processes.

Summary

The literature on the subject of the movement of organic materials in plants presents three main theories: (1) movement through the xylem due to a gradient of hydrostatic pressure, (2) movement through the phloem by diffusion and possibly accelerated by protoplasmic streaming, (3) mass flow through the sieve-tubes.

The first of these is not compatible with the anatomy of many plants, the second seems inadequate, the rates of diffusion and protoplasmic streaming appearing to be too slow to explain certain known rates of translocation and the third seems doubtful in view of the pressure gradients necessary to maintain flow through the constricted sieve-plate regions.

In place of these theories it is suggested that the osmotic system of PFEFFER, BLACKMAN and MÜNCH exists in the plant, the total phloem being

proposed as the channel for conduction with the majority of transport taking place in the cell walls.

In support of this proposition data from measurements and analyses on several plants are given.

Potato.—The phloem walls of the potato stolon occupy about 37 per cent. of the total phloem cross sectional area. These walls shrink to less than one-half of their original volume upon dehydration. Calculations indicate that rates of approximately one centimeter per minute through the total wall space are necessary to account for the accumulation of carbohydrates in the potato tuber.

Cucurbit.—Calculating from twenty collections of sap from pumpkin stems an average linear rate of flow of 0.81 centimeters per minute through the total phloem was recorded. Phloem walls constituted about 30 per cent. of the total phloem area in these stems. The walls shrunk 50 per cent. upon drying. Sieve-tubes occupied less than 20 per cent. of the total phloem area. Pores occupied about 8 per cent. of the sieve-plate area or 1.6 per cent. of the total phloem area. Using these values, calculations indicate that a pressure gradient of approximately 0.005 atmosphere would be necessary to force the volume of solution collected through one set of sieve-plates, or about 20 atmospheres per meter. Similar calculations show that a gradient of 6.1×10^{-5} atmosphere per centimeter would be needed to deliver this volume through a capillary tube having the same cross-sectional area as the water occupies in the phloem walls.

Further collections show that the initial rate of flow from cut cucurbit stems was about one centimeter per minute through the phloem. This rate drops off rapidly at first and then more slowly, approaching asymptotically a rate of 0.3 centimeter per minute. Calculations show that this is approximately the rate required to deliver the volume of solution necessary to form the fruits of these plants. Since rates of this magnitude cannot be accounted for on the basis of flow through the sieve-tubes it is suggested that the solution has passed through the phloem walls.

Calculations show that a rate of flow of 19 centimeters per hour would deliver enough materials through the phloem walls of Bartlett pear bark to account for the observed seasonal increase in dry weight.

A rate of 17 centimeters per hour would account for the transport of assimilate from the leaves of *Tropaeolum majus* and rates of 19.1, 15.0 and 13.5 centimeters per hour are calculated to be necessary in the phloem walls of the petiole of *Phaseolus multiflorus*.

Orange G solution was forced through the bark of several species of woody plants and rates of flow around 15 centimeters per hour, for a pressure gradient of one-third of an atmosphere per centimeter, were measured.

Acid dyes moved through the bark readily while basic ones were absorbed and failed to move.

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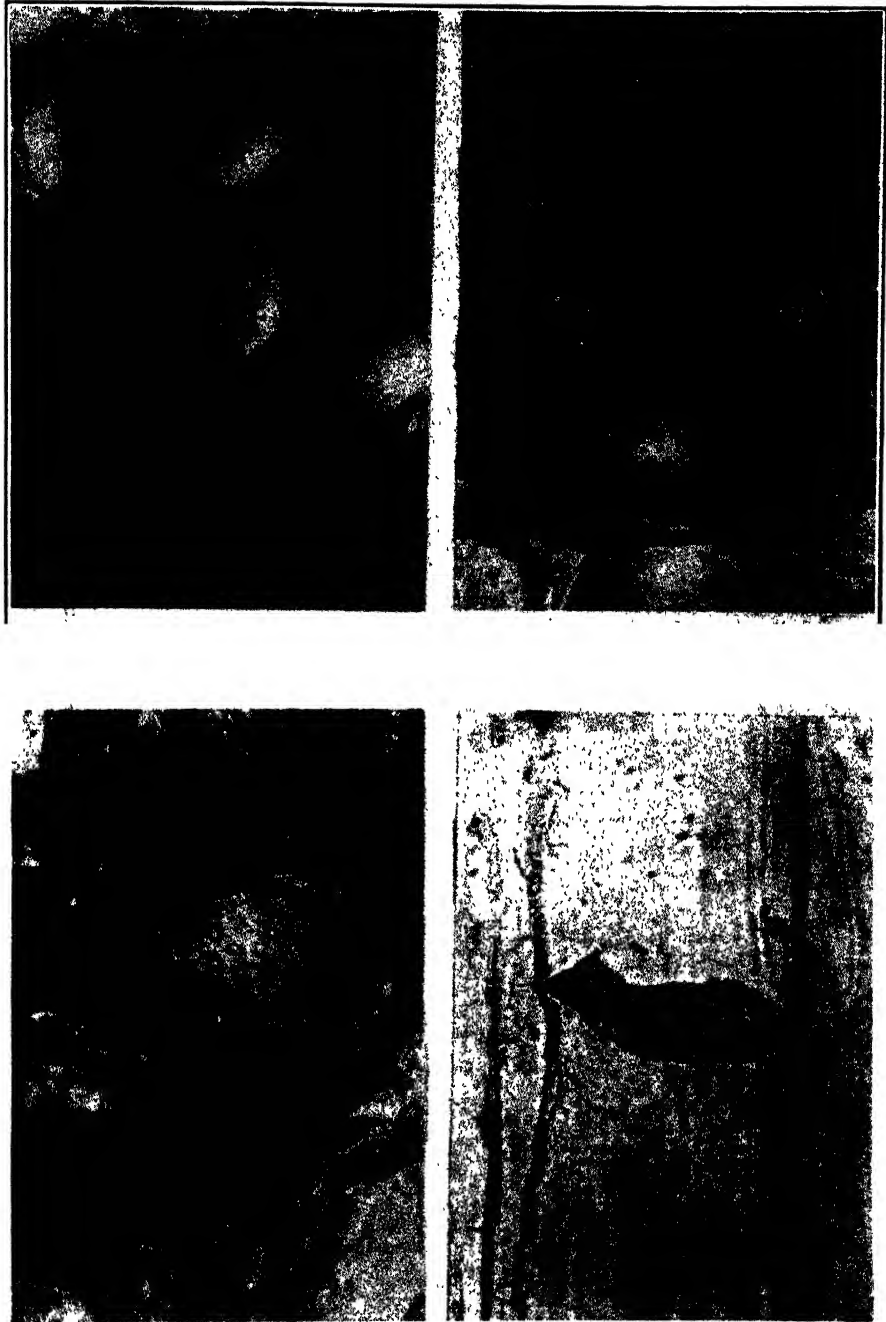
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EXPLANATION OF PLATES

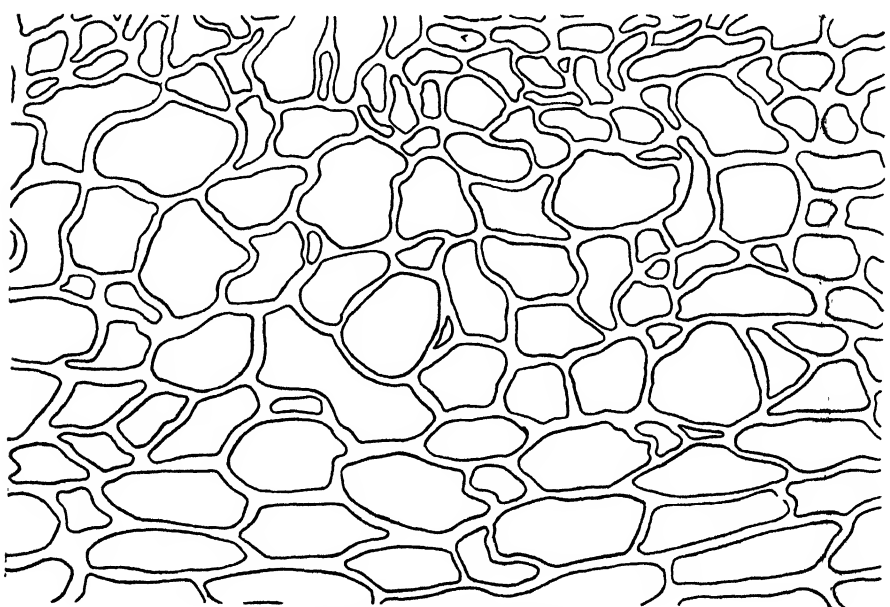
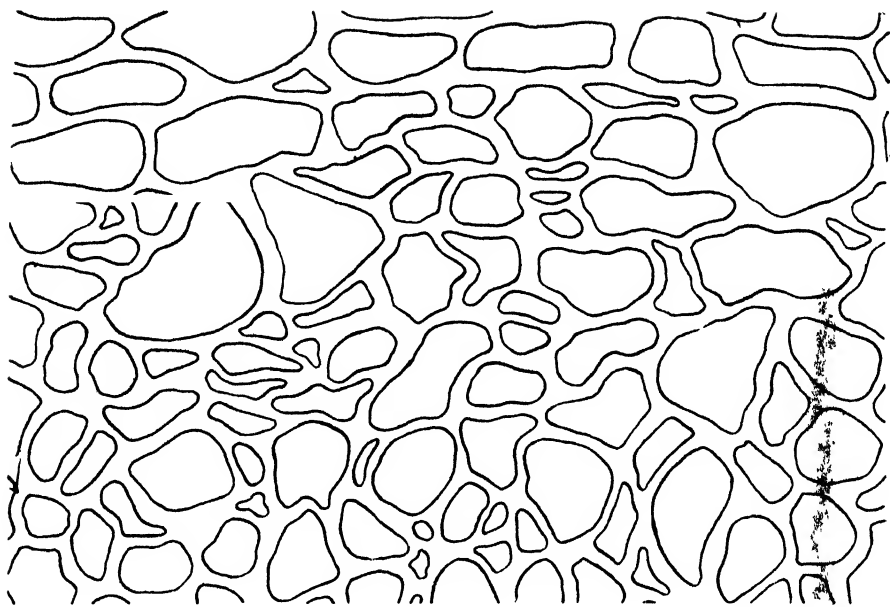
PLATE II

PLATE II shows photomicrographs of cucurbit sieve-plates as follows:

- (a) Upper left, cucurbit sieve-plate $\times 300$.
- (b) Upper right, cucurbit sieve-plates $\times 300$.
- (c) Lower left, cucurbit sieve-plate $\times 440$.
- (d) Lower right, cucurbit sieve-plate $\times 300$.



CRAFTS—TRANSLOCATION



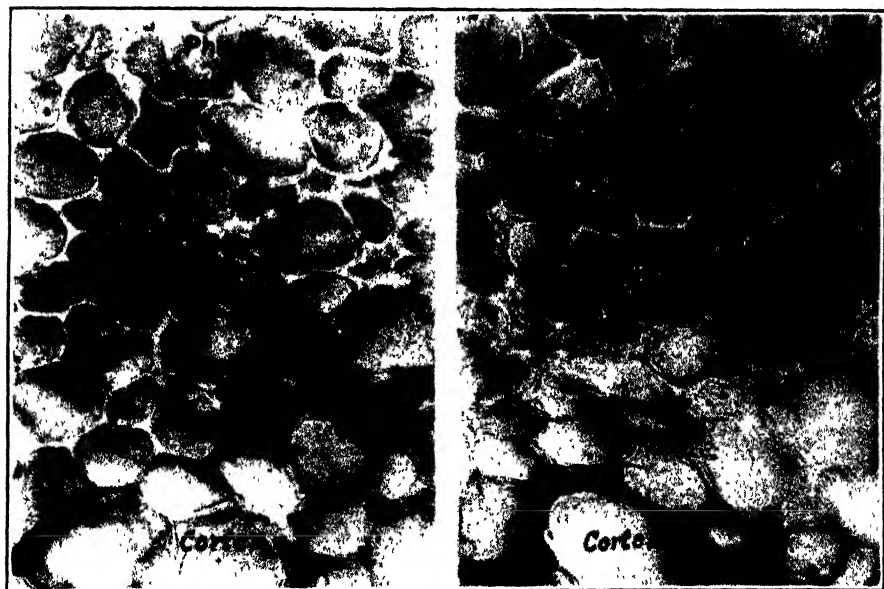
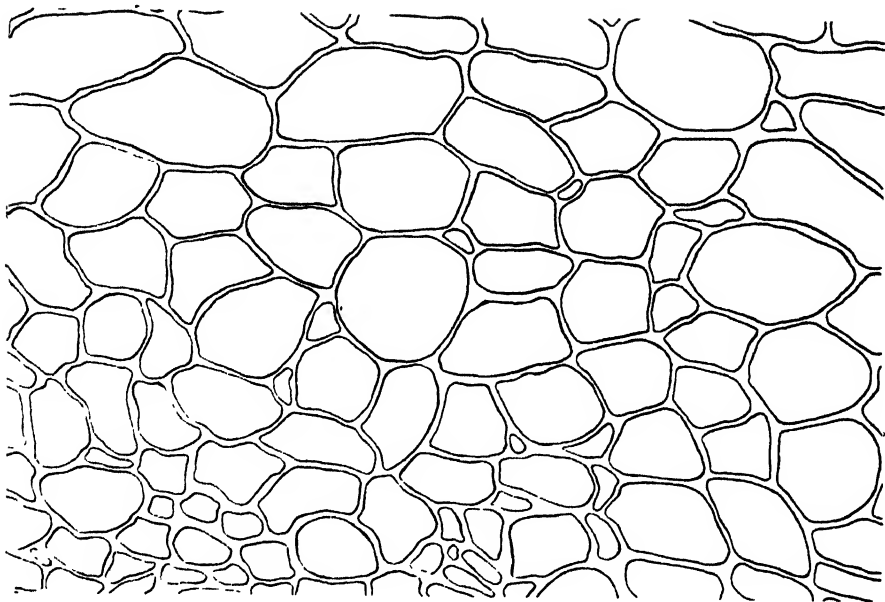
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PLATE III

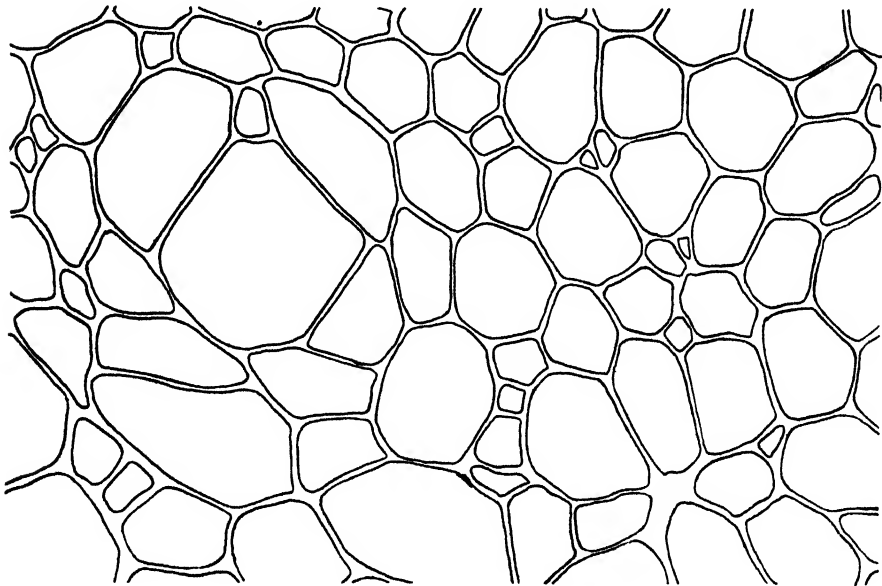
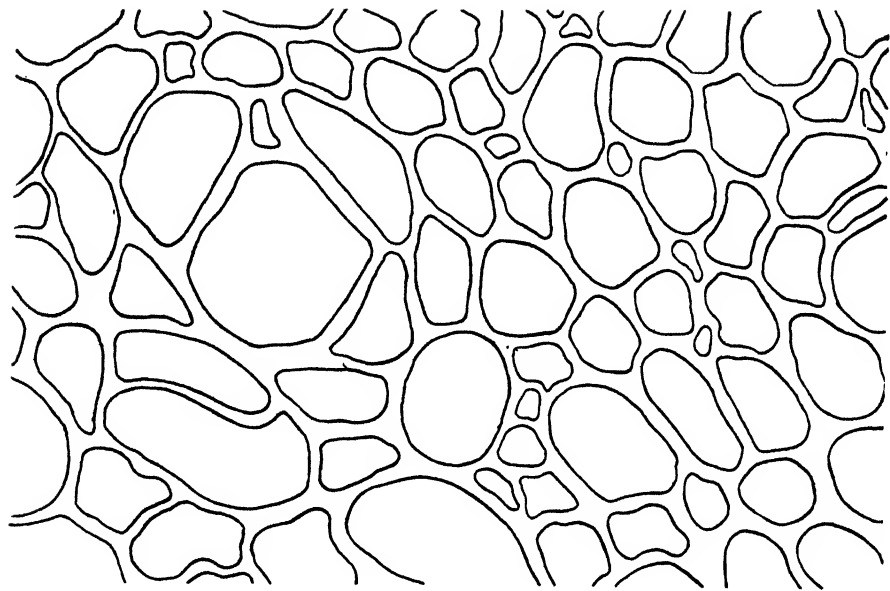
PLATE III shows (a) above, phloem of potato stolon $\times 560$, fresh hand section mounted in water. Below, (b) phloem of potato stolon $\times 560$, dehydrated in alcohol and mounted in balsam.

PLATE IV

PLATE IV shows (a) above, phloem of potato stolon $\times 560$, section air dried at 80° C.
Below, (b) phloem walls of *Cucurbita pepo* $\times 265$, fresh
sections mounted in glycerine.



CRAFTS—TRANSLOCATION



CRAFTS—TRANSLOCATION

PLATE V

PLATE V shows (a) above, phloem of pumpkin stem $\times 400$, fresh hand section mounted in water. Below, (b) phloem of pumpkin stem $\times 400$, same section as above, after air drying at 80° C.

FOOD RESERVES IN RELATION TO OTHER FACTORS LIMITING THE GROWTH OF GRASSES

L. F. GRABER¹

(WITH FOUR FIGURES)

The immediate applied significance of the pronouncements of LIEBIG (1840) with reference to mineral nutrition of plants, not only gave rise to profound changes in soil culture and crop husbandry, but also furnished the basis of the present fertilizer industry. The classic work of SACHS, PFEFFER, KLEBS and others on the organic nutrition of plants, however, has not been utilized to full advantage. Fairly recently (1918) KRAUS and KRAYBILL (12) have attempted to redirect attention to such studies, and many important contributions have been made by such workers as HARVEY (9), MURNEEK (14), NIGHTINGALE (17), ROBERTS (25), JANSSEN (11), LEUKEL (13), TIEDJENS (28), ALBERT (1), NELSON (16), WELTON (29), MORRIS (30), and others. Unlike mineral nutrients, the carbohydrates and other organic foods cannot at present be supplied to green plants in a commercial way. They must be elaborated by the living plant itself. The plant producer can vary cultural procedures, however, so that the formation, utilization, storage and destruction of such materials may be controlled or directed and crop production be more or less precisely regulated with reference to quality and yields. Such cultural practices include defoliations by cuttings, pruning and grazing; fertilization, especially with nitrogenous fertilizers; etiolation by shading or other limitations of light; irrigation and combinations of these treatments.

Food reserves as they are discussed in this paper may be regarded in a general way as those organic compounds which are synthesized and maintained in forms capable of being subsequently utilized by the plant in the performance of its various functions. They are labile materials. They involve so great a variety of organic compounds that present chemical technique and knowledge afford a clue of identification and understanding to but relatively few of them. They represent an accumulation or surplus of materials in excess of the immediate requirements of the plant for growth and maintenance. Their utilization is, essentially, most abundant in an environment favorable for growth extension and in those meristematic regions of the plant where the metabolism is most active. Generally, their storage occurs during periods when the accretions in dry weight are most rapid.

¹ Contribution from the Department of Agronomy of the Wisconsin Agricultural Experiment Station, Madison, Wisconsin. Published with the approval of the director.

With the progressive enlargement of the photosynthetic area in plants, sufficient new foods are manufactured not only to provide for growth extensions but also to provide for accumulations and storage of such foods for subsequent growth. However, when photosynthesis is interrupted by frequent and complete removals of the green parts, the plant may not have an opportunity to replenish the losses of previously stored foods which were used up in its regenerative activity. Especially is this true when regeneration of new growth is stimulated by mineral nutrients, moisture and temperature, and when photosynthesis is further curtailed by shading or other forms of light deficiency. Under such circumstances the growth rate soon declines and although the levels of reserves might be sufficient to maintain the existence of the plant for some time, death eventually ensues.

The data presented in this paper, represent an attempt to correlate certain cultural procedures with the utilization and accumulation of organic foods. There is a general recognition of the ready response of plants to fertilization, moisture and to changes in temperature and light conditions, but when cultural practices involve defoliation, the responses are usually delayed and are often ascribed to some other cause even though the effect on the internal environment may be pronounced and be clearly reflected in subsequent growth. Thus alfalfa (*Medicago sativa* L.) with an abundance of stored foods may yield more during one season of growth if cut frequently rather than infrequently, but the ill-effects of frequent cutting are usually pronounced the following season. Similarly, blue grass (*Poa pratensis* L.) may be grazed closely and early for a season or more with good results, but the decline in its productivity is certain, even though not readily recognized because of wide variations in seasonal conditions, and because of the growth of other plants among the blue grass, which have not been affected so seriously by the grazing treatment. Food reserves are so intimately associated and inter-related with all other limiting factors of growth that it is often difficult to differentiate from drought, winter injury, infertility, or some other factor or factors, the particular effect a lack of such reserves may have played in developing any specific condition under consideration.

Reserves in relation to morphology and growth habits

The wide variations in the responses of agronomic plants to specific practices of top growth removal are correlated with some of their general characteristics of growth such as the duration of the succulent period and the structure and arrangement of the photosynthetic parts. PIERRE and BERTRAM (23) have studied the storage of organic foods as they affect the tops and roots of kudzu (*Pueraria thunbergiana*), a leguminous vine which is being utilized for forage in southern United States. Accord-

ing to PIPER (24) this is a very late blooming plant with a long period of succulent growth, maturing only occasionally in the latitude of Washington, D. C. PIERRE and BERTRAM report that, in Alabama, "the reserve starch and nitrogen were found to be less than half as much in the roots of plants receiving six cuttings per season for two years as in roots of plants receiving four or a less number of cuttings." During a period of two years, the weight of the roots of kudzu increased 1250 per cent. when the top growth was cut but once annually near maturity, while with six cuttings of immature top growth the roots actually decreased in weight. In the third year, the yield of top growth from the plants cut six times was only about one thirty-third of that obtained from plants cut but once annually. Other cutting treatments were tried but these will suffice to illustrate the marked responses of the kudzu plant to cultural treatments which affect the food reserves. With a relatively long period of succulence and a rapid rate of growth, several cuttings during the growing season apparently give little opportunity for sufficient accumulation of stored foods to assure the maintenance of a desirable productivity in this perennial.

The alfalfa plant has a high metabolic activity, with great ability for regeneration; and its reserves are rapidly reduced by frequent cuttings. It is obvious, on the other hand, that the reserves of small short plants such as blue grass (*Poa*) or creeping bent grasses (*Agrostis*) would be much less affected by frequent mowing with a field mower or a lawn mower, partly because of the slow recovery in vegetative extension, and particularly because of the greater proportion of the photosynthetic area remaining after each cutting. Such green remnants of herbaceous plants have the capacity to replenish, in part, the losses of reserves sustained by frequent cuttings, close grazing or other forms of removal. Under field conditions a much greater proportion of the photosynthetic area of tall, erect plants is removed by cutting or by close grazing than is true of plants whose leaves diverge largely from short branches of rhizomes or stolons, or of plants with short stems and a decumbent or rosette type of growth where the photosynthetic area consists largely of basal leaves near the soil surface. The general structure of the plant, therefore, has an important bearing on its capacity to maintain sufficient reserves for its survival under exacting grazing or cutting treatments. The persistence of blue grass (*Poa*), plantain (*Plantago*), dandelions (*Taraxacum*), purslane (*Portulaca*), creeping spurge (*Euphorbia*), and many other prostrate plants which maintain themselves in fields, lawns and pastures is thus explained in part. As shown by GRABER, NELSON, LEUKEL and ALBERT (8) twenty-two close cuttings with a lawn mower did not kill blue grass (*Poa pratensis*) though it

lessened its productivity, while only nine such cuttings resulted in death to nearly all the plants of alfalfa. Close cutting of blue grass with a lawn mower reduced subsequent productivity much more than "tall" cutting with the same implement.

In experiments conducted by the writer (7) during 1929 with blue grass seeded in 1928, the simple expedient of setting the lawn mower to cut "high" produced a thick turf free of weeds in contrast to one which was thin and weedy due to close cutting with the same frequency. An uncut remnant of one and one-half inches of the foliar parts of grasses is decidedly more effective in maintaining a satisfactory level of reserves than a half inch of such growth. This is not only significant in the maintenance of blue grass lawns where density of growth and freedom of weeds are factors which add to the value of the turf, but it has applications in the management of pastures. ALDOUS (2) indicates that the unfavorable effects of frequent clipping of prairie grasses are partially eliminated by increasing the height of clipping. STAPLEDON and MILTON (26) report that cocksfoot (*Dactylis glomerata*) plants cut frequently to a six inch level have considerably outyielded those cut to ground level. The development of tillers and roots was also much greater under the less drastic system of cutting.

The Hohenheim system

Recently, investigations have been started in America to determine the merits of the Hohenheim system of pasture management. In the main, this German system involves heavy fertilization (with mineral and especially nitrogenous fertilizers) of permanent pastures which are grazed closely at intervals throughout the year. It is evident that the success of this system of rotational grazing must make provision for the maintenance of a sufficient surplus of carbohydrates. With the stimulus of a liberal supply of nitrogen, rapid growth not only consumes organic foods for energy and cell wall materials, but the carbohydrates are utilized in the synthesis of large amounts of proteins. Heavy nitrogenous fertilization of blue grass also makes possible the stimulation of top growth at the expense of root growth, especially where grazing or cutting is sufficiently delayed to produce etiolation effects in the buds and meristematic regions of the leaves. However, by avoiding extremely early and close spring grazing and by proper extension of the intervals between grazing, the maintenance of an adequate carbohydrate reserve is readily made possible. The agronomic importance of ascertaining the responses of pasture grasses to varying "periods of rest" in rotational grazing is very evident especially under conditions of a very favorable soil and climatic environment.

While judicious fertilization may for a time stimulate the utilization of carbohydrate reserves, it is not amiss to appreciate that such stimulation

may also accelerate their accumulation when the plant is given an opportunity for such storage. Fertilization often trebles the productive capacity of blue grass and other pasture grasses grown on soils deficient in general fertility. Such enhanced potentialities in the synthesis of foods must greatly aid the green remnants which escape grazing to maintain a productive level of reserve foods in such grasses. It is primarily when these remnants are kept sufficiently reduced by close grazing or cutting treatments that the stimulation in growth from judicious fertilization is apt to hasten the decline of the reserves to a degree of serious curtailment of subsequent productivity.

Survival values and reserves

Grasses in lawns, golf courses, and pastures may exist for many years even though they receive treatments which are not conducive to the maintenance of a high reserve content. It is always to be recognized that plants with low supplies of organic foods may survive even though their capacity for survival is greatly diminished. In extreme cases, however, the maintenance of special external conditions is necessary for such survival and the constant watering and careful fertilization of golf greens and closely cut blue grass lawns are examples of such effort. Such special conditions augment the limited root growth of low reserve plants and aid the synthesizing areas which escape removal, in maintaining a level of reserve foods sufficient for the survival of the plants. Under field conditions, however, the limitations of root growth, and the modifications of the internal environment resulting from low reserves may reduce the absorptive capacity of a plant so greatly and may so increase its susceptibility to drought, winter injury, weed encroachments, insect injury and other hazards as to jeopardize its permanence. Such secondary effects often intensify the influence of low reserves on the productivity of plants, and since they appear so obvious, they may cloak this primary cause of many forms of deterioration in economic plants.

Observations made by the writer (3) in southwestern Wisconsin, where permanent blue grass grazing lands have been grazed for thirty years or more, have revealed that insect injury from white grubs (*Phyllophaga* spp.) and weed infestations appeared much more abundant in pastures which had been prematurely over-grazed or which were deficient in fertility or where soils were thin and dry due to outcroppings of limestone or flint, or where combinations of such conditions occurred. While these factors may encourage insect infestations, they also limit the amount of subterranean growth, and, in general, where such growth was least, insect injury was most abundant. The survival of economic plants, their productivity and their competitive efficiency under field conditions are dependent upon

many inter-related factors but organic reserves are among the most important.

The experiments reported in this paper show some of the correlations between agronomic practices and food reserves as they affect productivity under various soil environments and as they affect the ecological relationships of such plants under field conditions. KRAUS and KRAYBILL state that "a plant at any particular time represents the result of all the environmental forces acting upon it, and it is either in a state of equilibrium with such forces or in a state of becoming so adjusted." The adjustment of plants to those conditions which are brought about by such cultural treatments as grazing, cutting, fertilization and irrigation, is highly complex and must be approached from a study of both internal and external relations.

Cutting treatments in relation to productivity of blue grass with and without fertilization

EXPERIMENT I

A strip of land on the University Farm, Madison, Wisconsin, classified as Miami silt loam, was summer fallowed during 1925, and up to July 22, 1926, on which date it was sown with blue grass seed (*Poa pratensis* L.) at the rate of 100 pounds per acre. By fall a thick uniform growth of grass had developed to a height of about four inches. No flower stalks appeared that season nor was the grass cut. On April 11, 1927, the grass was rolled twice with a three-ton roller so as to produce a smooth surface for uniform mowing with a lawn or field mower. The area was divided into four plats of 1/35 of an acre, each designated by the letters A, B, C, and D. Plats A and C were not fertilized during the period of the experiment, but plats B and D received the following applications expressed in pounds per acre:

March 18, 1927—	220 pounds of super-phosphate (16 per cent. P_2O_5)
	140 pounds of potassium sulphate (40 per cent. K.)
	70 pounds of sodium nitrate (16 per cent. N.)
May 26, 1927—	140 pounds of ammonium sulphate (20 per cent. N.)
July 30, 1927—	200 pounds of ammonium sulphate (20 per cent. N.)
May 15, 1928—	200 pounds of ammonium sulphate (20 per cent. N.)
July 12, 1928—	350 pounds of ammonium sulphate (20 per cent. N.)

Thus, a total of 79 pounds of elemental nitrogen per acre was applied in 1927 and 110 pounds in 1928. The soil was naturally abundant in lime and in a fairly high state of fertility. The summer fallowing which preceded seeding probably increased the available fertility very considerably.

In 1927, two different cutting treatments were employed on the fertilized and unfertilized areas. Plats C (unfertilized) and D (fertilized) were cut six times with a lawn mower (set to mow about one inch above the

surface of the soil) on April 6, 29, May 11, 27, June 8 and 24, 1927. Plats A (unfertilized) and B (fertilized) were cut only once with a field mower when near maturity on June 24, 1927.

In 1928, the plats or representative portions of them were cut twice; once at maturity on July 7, 1928, and again on August 31, 1928. All cuttings were weighed in the green state and a representative sample of from 500 to 1000 grams was taken for a determination of moisture content so as to arrive at the dry weights on the basis of oven-dried grass. No attempt was made to accomplish complete desiccation although the samples were kept in a drying room for periods of from four to six days at temperatures ranging from 100° F. to 110° F. which, it is believed, provided dry-

TABLE I

COMPARATIVE AMOUNTS OF BLUE GRASS (*Poa pratensis* L.) OBTAINED FROM SIX CUTTINGS AT IMMATURE STAGES OF GROWTH AND ONE CUTTING NEAR MATURITY WHEN GROWN ON FERTILIZED AND UNFERTILIZED SOIL. BLUE GRASS SOWN JULY 22, 1926. RESULTS EXPRESSED IN POUNDS OF OVEN-DRIED GRASS PER ACRE

PLOT	FERTILIZATION	DATES AND AMOUNTS OF OVEN-DRIED TOP GROWTH REMOVED DURING 1927						
		4/6	4/29	5/11	5/27	6/8	6/24	TOTAL
C	None	244	255	332	156	133	149	1269
A	None						2930	2930
D	Abundant	146	203	342	183	236	266	1376
B	Abundant						3563	3563

TABLE II

THE INFLUENCE OF CUTTING TREATMENTS IN 1927 ON THE PRODUCTION OF BLUE GRASS DURING 1928 EXPRESSED IN POUNDS PER ACRE

PLOT	FERTILIZATION	DATES AND AMOUNTS OF OVEN-DRIED TOP GROWTH REMOVED DURING 1928				
		7/7/28			8/31/28	TOTAL FOR 1928
		LEAF GROWTH	FLOWER* STALKS	TOTAL	LEAF GROWTH ONLY	
C	None	448	152	600	603	1203
A	None	1443	279	1722	1037	2759
D	Abundant	1609	353	1962	2674	4636
B	Abundant	2630	523	3153	3315	6468

* Flower stalks include leaves diverging at nodes.

ing to a fairly uniform state. All yields were calculated on the basis of such oven-dried hay, and are given in tables I and II.

RESULTS.—The abundant rainfall, measuring 12.41 inches, during April, May and the first 24 days of June, 1927, benefited the growth of the blue grass as did also the favorable conditions of the soil on which it grew. However, when the cumulative effects of photosynthesis were diminished by frequent cuttings, the production of dry matter during a given period was

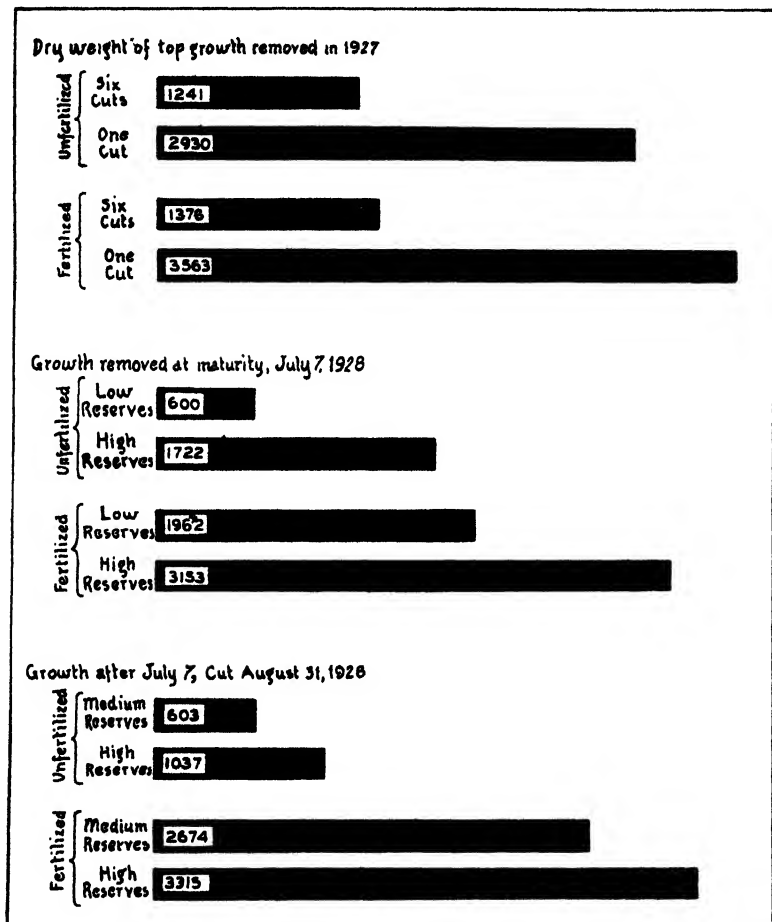


FIG. 1. Productivity of blue grass with relation to food reserves and fertilization. Six cuttings of blue grass made with a lawn mower in 1927 on April 6, 29, May 11, 27, June 8 and 24, not only gave less than half the amount of dry matter obtained with one cutting taken near maturity on June 24, 1927, but greatly reduced the productivity of the blue grass the following year as measured on July 7 and August 31, 1928. Amount of top growth removed is expressed graphically on the basis of pounds of oven-dried grass per acre.

greatly reduced. Thus, on the unfertilized soil, six cuttings of immature blue grass (figure 1) yielded but 43 per cent. of the amount of dry matter obtained from one cutting near maturity (on June 24) while with fertilization, six cuttings produced only 38 per cent. of the amount of dry matter obtained with one cutting.

The productive responses of blue grass to the fertilizations made on March 18 and May 26, 1927, were much less pronounced when six cuttings were taken during the spring and early summer period (up to and including June 24, 1927) than when one cutting was taken near maturity on June 24. Comparing the amount of growth removed from the fertilized and unfertilized plats of blue grass given identical cutting treatments, fertilization increased the yield (table I) of blue grass cut six times, 11 per cent. and that cut once, 21 per cent.

During the last six days of June and in July and August, the rainfall was only 2.43 inches so that little growth occurred during this period but in September and October when 10.45 inches of rain fell the blue grass made considerable recovery. This growth was not removed, which gave excellent opportunity for fall storage of organic foods. However, the six early cuttings prior to June 25, 1927, had a profound effect on the productivity of the blue grass in 1928. This is shown by data in table II and graphically by figure 1.

Plats B and D were fertilized again with ammonium sulphate in 1928 on May 15 and July 12, with applications sufficient to apply 110 pounds of total elemental nitrogen per acre, while plats A and C remained unfertilized. All plats were cut when fully mature on July 7, and again on the last day of August. This made possible a measurement in 1928 of the residual effects of the cutting treatments applied in 1927 on the productivity of the grass grown on the fertilized and unfertilized soil.

Without fertilization the blue grass with "high" reserves produced on July 7, 1928, nearly three times the amount of dry matter (figure 1) obtained from blue grass with "low" reserves. With fertilization the yields of blue grass with "high" reserves were 1.6 times those obtained from the blue grass "low" in reserves. Low reserves imply limited root growth, limited absorptive capacity and limited drought resistance, which conditions may well have been obviated, in part, by heavy fertilization. The designations "low" and "high" with reference to reserves are based only on previous cutting treatments and not on chemical analysis. These comparative terms are used to simplify the discussion.

It is not assumed that the deficiencies in reserves which were occasioned by the six cuttings in 1927 were alone responsible for the reduced yields on July 7, 1928, but in all probability, they were of major importance. Cutting treatments which reduce food reserves tend to retard subterranean

growth and may well lower the absorptive capacity of the plants. The marked responses from fertilization in 1928, resulting in increases of dry matter from 234 to 385 per cent., offer a striking contrast with increases of only 11 to 21 per cent. in 1927. In part, at least, such increases indicate changes in available fertility (particularly nitrogen) and losses of nutrients from the removals of grass in 1927.

The abundant rainfall during the latter part of June and in July and August, 1928, made possible another measurement of the productivity of blue grass on August 31, 1928. The fact, however, that the blue grass "low" in reserves was allowed to mature before cutting in 1928 made possible some re-establishment in the supplies of stored foods of the blue grass cut six times in 1927. For this reason, the designation, in figure 1, for the growth of blue grass after July 7, was changed from "low reserves" to "medium reserves" to approximate the probable situation of the organic foods in relation to the yields of blue grass determined on August 31. The growth removed at this time was all leaves, no flowering stalks having developed after cutting on July 7. While the grass with high reserves again yielded most, whether grown on fertilized or unfertilized soil, the differences in yields with reference to reserves are not as pronounced on August 31, 1928, as they were on July 7, 1928. Allowing blue grass with "low" reserves to fully mature apparently resulted in sufficient recuperation of the organic food surplus to increase the general metabolism and subsequent summer productivity. Whatever this may have involved in the way of additional root and rhizome development or in a greater absorptive capacity of the subterranean parts or in a greater release of energy for more rapid growth, such conditions were not sufficient to restore the full productivity of the plants as expressed by the blue grass with "high" reserves.

Reserve foods and fertilization.—The yields illustrated in figure 1 are indicative of the fact that although the soil may be abundantly supplied with available nitrogen, phosphorus, potash and all other requisites of growth, the plant may be deficient in organic materials for the greatest expression of its productive capacity. The productivity of blue grass with relation to nitrogenous fertilization is dependent, not only, upon adequate supplies of moisture, phosphorus, potash and other limiting elements within the soil, but upon the reserve foods within the plant which are also limiting factors of growth. A relatively greater response from nitrogenous fertilization occurred with blue grass having low reserves than with similar fertilization of blue grass where the reserves had not been lowered by previous cutting treatments. Fertilization during 1927 and 1928 increased the yields (taken on July 7, 1928) of blue grass with low reserves 327 per cent. in comparison with only 183 per cent. increase from the same fertiliza-

tion of blue grass with high reserves. On August 31, 1928, the increase from fertilization was 443 per cent. for the grass then having medium reserves compared with 320 per cent. for the grass with high reserves. That an explanation of such variations in the responses of high and low reserve grass to similar fertilization may rest, in part, on a greater capacity for the absorption and utilization of limited supplies of soil nutrients by plants having abundant food reserves is indicated by the recent work of STOKES, LEUKELE and BARNETTE (27). They found in the crowns of Napier grass (*Pennisetum purpureum*) which had been irrigated for three years with sewage effluent, that the quantities of dry matter, various carbohydrate compounds, total available carbohydrates and total nitrogen were approximately three and one-half times that of similar materials in the crowns of non-irrigated plants. The percentages of these organic compounds were quite similar, however, in the plants with and without irrigation. "It is apparent," they state, "that the continued large yields of Napier grass silage from the irrigated plats after the discontinuation of the irrigation with sewage effluent, cannot be explained alone on the basis of a greatly increased soil fertility of the irrigated plats nor to water alone as shown by the yields of the city water irrigated plat." The production of a larger plant system through irrigation with sewage effluent increased the elaboration of organic foods, a condition "reflected in the increased growth of the lower storage parts of the plants." Analysis of crowns subsequent to the discontinuance of irrigation, indicated that with a more extensive absorptive system and with more abundant carbohydrate reserves, a greater efficiency in the utilization of plant nutrients (particularly nitrogen) occurred.

Growth recovery.—The growth recovery of the blue grass on the fertilized plats after cutting on July 7, 1928, was much more rapid and uniform at the outset with the grass having been cut six times in 1927 than with that cut but once in 1927. During the latter part of July, 1928, the former plats of blue grass were uniformly green while the latter still appeared brown and somewhat patchy, the new growth not being thick enough to hide uniformly the dead leaves of the old stubble. On the unfertilized plats a somewhat similar contrast was apparent but was much less pronounced. It is well known that thick stands of the second year's growth of sweet clover (*Melilotus alba*) often fail to recover after late cutting. Here the young shoots and axillary buds at the lower nodes of the stems have been seriously injured by etiolation from the tall dense growth above but when this legume is grazed or clipped at immature stages, sunlight is provided for the basal leaves and buds and they continue to develop.

In this experiment, the growth of blue grass with high reserves and high fertility was so rapid and so abundant during early summer that the devel-

opment of the buds on the rhizomes or leaves diverging from the upright branches of the rhizomes was largely inhibited by the competition of the dense growth above. The basal portions of the older leaves were largely killed by the effects of etiolation and rather sudden light exposure after cutting. These factors involved delay in recovery because subsequent regeneration occurred largely from dormant buds on the rhizomes or the uninjured leaves of their branches. With less abundant top growth during spring and early summer, etiolation occurred to a much less extent and the new leaf growth was able to elongate in competition with the older growth so that when the latter was removed the leaves which partly or completely escaped mowing were in a state of growth and continued to develop after cutting.

When comparative yields on August 31, 1928, are considered, it is apparent that in this experiment, the productive capacity of blue grass well supplied with reserve foods and with abundant fertility was sufficient to compensate for the losses sustained by slow recovery resulting from the retarding effects of previous heavy growth. Such delayed recovery would, of course, prove very disadvantageous in the maintenance of lawns and of other turfs where uniformity is desired and it is a factor of importance with pasture management where heavy nitrogenous fertilization is practiced. When cutting is sufficiently delayed beyond maturity the desiccation of the old growth may so reduce its competitive effects that the development of new buds and leaves occurs and recovery after cutting is more rapid.

Ecological considerations.—The amounts of foreign growth in the plats during 1927 was very small. Just before cutting the blue grass on August 31, 1928, the weed growth was considerable, and it was removed from part of each plat (1/70 acre) with each species being counted as summarized in table III. On the unfertilized soil, the weeds were ten times more abundant in the blue grass cut six times in 1927 than were cut once that year. Fertilization, however, was a very potent factor in preventing the ingress of foreign plants. The weeds in the fertilized blue grass cut six times in the previous year were only about one-seventh of the number found in similar blue grass growing on unfertilized plats. The failure of the fertilized plats of blue grass with "high" reserves to recover rapidly gave purslane and barnyard grass a vigorous start before the grass recovered sufficiently to become a competitive factor. Both of these weeds in absence of initial competition were favored by fertilization and abundant rainfall which occurred during the summer of 1928. Purslane was present only in the fertilized plats of "high" reserve blue grass where delay in growth recovery occurred after cutting on July 7, 1928. Barnyard grass and pigweed were most abundant in these plats while other weeds were absent or

TABLE III

NUMBER OF WEEDS IN PLATS OF BLUE GRASS OF ONE-SEVENTIETH OF AN ACRE AS
AFFECTED BY FERTILITY, CUTTING TREATMENTS AND ORGANIC FOOD
RESERVES. DETERMINATIONS MADE ON AUGUST 30, 1928

SPECIES	SIX CUTTINGS 1927 ONE CUTTING JULY 7, 1928		ONE CUTTING JUNE 24, 1927 ONE CUTTING JULY 7, 1928	
	FERTILIZED	UNFERTILIZED	FERTILIZED	UNFERTILIZED
Witch grass (<i>Panicum capillare</i> L.)	8	535	26	8
Foxtail (<i>Setaria glauca</i> L.) (<i>Setaria viridis</i>)	48	260	6	44
Purslane (<i>Portulaca oleracea</i>)	0	0	280	0
Barnyard grass (<i>Panicum crusgalli</i> L.)	15	11	158	10
Horseweed (<i>Erigeron canadensis</i> L.)	0	234	0	22
Others	92	83	20	45
Total	163	1123	490	129

sparse. Foxtail (*Setaria*), witch grass (*Panicum*), dandelions (*Taraxacum*), cinquefoil (*Potentilla*), horseweed (*Erigeron*), and field sorrel (*Oxalis*) were conspicuous in the unfertilized plats of the blue grass given six cuttings in 1927.

While an invasion of weeds occurred in some of the plats during the summer of 1928, the only foreign growth which was prevalent in 1929 was white clover (*Trifolium repens*). Neither weeds nor clover appeared in any of the plats heavily fertilized with nitrogen, but in the unfertilized plats especially of the blue grass cut frequently in 1927 white clover was very abundant. The generous rainfall during the summer of 1928 made it possible for the dormant seeds in the soil to establish seedling plants of white clover which, in turn, were protected by an abundance of snow during the following winter. The soil was favorable for growth of this legume so that the external environment made possible its sudden appearance in the plats of blue grass which had not been fertilized.

The prevalence of plants other than blue grass in the plats of this experiment is somewhat analogous to the general situation observed in the pasture lands of the blue grass region of southwestern Wisconsin. The weed population of grazed blue grass may vary widely in kind from blue grass given various cutting treatments, but there are some noteworthy similarities. In general, it can be said that those factors which encourage the growth of blue grass tend to retard the ingress of weeds or clovers in

such blue grass. Thus, in the pastures of southwestern Wisconsin both weeds and white clover are generally very sparse where soils are fertile and retentive of moisture, and where the blue grass is grazed judiciously and is well supplied with reserve foods. See fig. 2 for influence of grazing on the flora of blue grass pastures. This condition tends to maintain a heavy



FIG. 2. Grazing practices influence flora of blue grass pastures. Close, early spring grazing has lowered the reserves and the competitive efficiency of the blue grass on the hillside (to right) and a heavy growth of unpalatable ragweeds (*Ambrosia*) has occurred in the pasture while with deferred and lighter grazing (left) these weeds are absent. The portions of the pastures shown above have never been plowed and although hilly the soil is regarded as being in a fairly fertile condition.

sod and during the spring season, at least, an abundant top growth, both of which are effective in preventing the entrance of foreign growths. It is not meant to imply that blue grass with optimum conditions for growth is able to exclude all forms of weed growth, but rather to indicate that when the nitrates and other mineral elements of the soil, and the organic reserves of grasses in grazed pastures are maintained in an optimum state, the competition of the blue grass is usually sufficient to prevent or retard the ingress of many undesirable plants as well as desirable ones.

The requirements of white clover for lime, phosphate, and potash are pronounced and when these nutrients are deficient in soils of grasslands, this clover is usually sparse and weeds are often abundant. The preva-

lence of this legume in southwestern Wisconsin is also influenced by climatic conditions of both winter and summer. It is probably less sensitive to drought and surely much more susceptible to winter injury than blue grass.

Many weeds such as ragweed (*Ambrosia*), dandelion (*Taraxacum*), vervain (*Verbena*), thistle (*Cirsium*), yarrow (*Achillea*), and others have a distinct advantage in competing with early and closely grazed blue grass. Such weeds may prevail because of their drought resistance or because of their general structure or inedibility or unpalatability or combinations of these characteristics which permit a partial escape from grazing even when pastures are heavily grazed. They have the advantage of being able to provide for permanence by forming seed and by maintaining reserves of organic foods. Tall plants which are palatable and with foliar parts located so as to be readily grazed, often disappear or fail to become established because with constant grazing they may not be able to form seed or maintain an adequate supply of reserve foods for their survival.

When grasses are permitted to make considerable growth before cutting or grazing, they offer greater resistance to the establishment of seedlings of foreign plants than if cut frequently. As shown by GRABER (4, 5, 6) viable seeds of legumes sown on dense sods or where accumulations of old grass had occurred, failed to establish plants; but when such old grass was burned off, or when the sod was scarified so as to provide soil contacts for the viable seeds, the seedlings were successful, if soil, climatic and other conditions were adapted to their requirements for growth. The opportunity for plants to establish themselves by seed dispersal in permanent grasslands is dependent upon factors which influence the possibilities of soil contacts for the seed as well as an environment sufficiently favorable for subsequent development. Such possibilities are often enhanced by the frequent removals of accumulations of top growth by close grazing or cutting.

There are, of course, many other external factors, such as climatic conditions, the prevalence of insects which feed on subterranean growth, the acidity of the soil, its content of nitrogen (see fig. 3) and other mineral components, its moisture holding capacity and its drainage, all of which affect the flora of permanent grasslands; but the factors which affect the internal environment of pasture grasses are also important. Under the complexities of field situations organic food reserves of blue grass as affected by cultural treatments are not only correlated with other factors which affect the quantitative responses of this plant, but such surplus foods also have an ecological significance in maintaining a high survival value and a competitive efficiency when the growth of this important grass is jeopardized by climatic, insect and weed hazards.

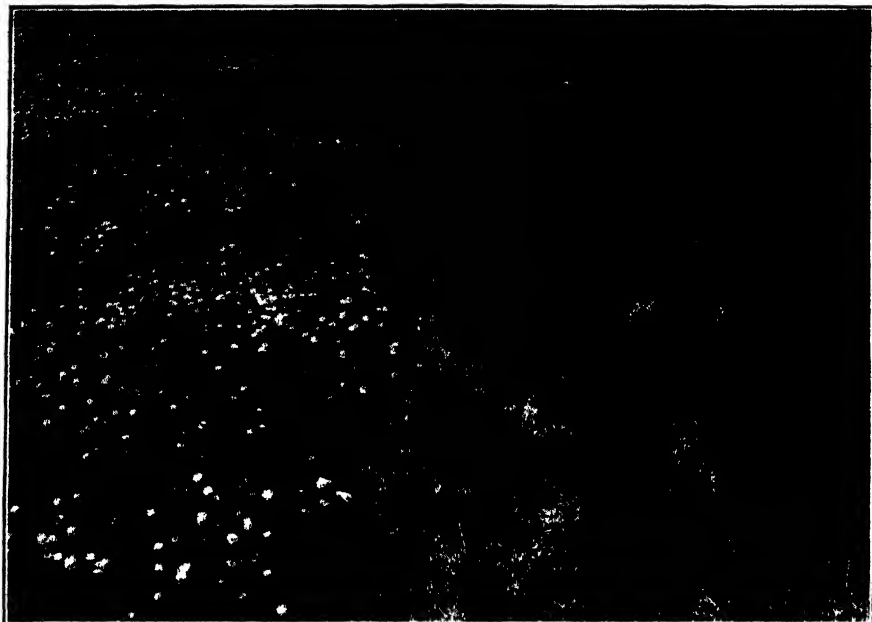


FIG. 3. Nitrogenous fertilization curtailed growth of white clover and weeds in blue grass. A volunteer growth of white clover occurred only in the unfertilized blue grass (left) while its absence in the adjacent plot resulted from abundant applications of nitrogenous fertilizers. Photo taken June 25, 1929.

Nitrogen relations

EXPERIMENT II

On July 14, 1925, blue grass (*Poa pratensis* L.), red top (*Agrostis alba* L.), fescue (*Festuca rubra fallax*), and timothy (*Phleum pratense*) were each sown separately in four plats of one twenty-fifth of an acre each of a Miami silt loam soil which had been summer fallowed for two years. A thick dense growth of these grasses resulted that year, and they attained a height of from three to five inches in the fall of 1925. No cuttings of top growth were made in 1925. Beginning in 1926 one-half the area of each plat was top dressed with applications of fertilizer on the dates and in the amounts per acre as follows:

April 19, 1926—500 pounds of 4-16-6 fertilizer (4 per cent. N, 16 per cent. P_2O_5 , 6 per cent. K_2O).

July 13, 1926— 500 pounds of 4-16-6 fertilizer.
3000 pounds of dolomitic limestone.

March 18, 1927—125 pounds of ammonium phosphate (10.63 per cent. N).

May 20, 1927—125 pounds of calcium cyanamid (21.73 per cent. N).

July 30, 1927—200 pounds of ammonium sulphate (20 per cent. N).

May 12, 1928—125 pounds of ammonium sulphate (20 per cent. N).

The fertilized and unfertilized half of each plat was divided into two equal parts of 1/100 of an acre each and a plat of each grass on the fertilized area and one on the unfertilized area was cut once with a field mower when at or near maturity on July 10, 1926. The remaining two plats of each grass were cut (with a mower) on May 22, June 12, July 10, and August 18, 1926. The yields of the grasses with these cutting treatments are given in table IV.

In 1927, the plats of grass cut four times in 1926 were given a more intensive cutting treatment by close mowings (about one inch above soil surface) with a lawn mower on April 6, May 2, 21, 30, June 8, 24, and October 28. The plats of grass cut only once in 1926 were again cut only once on June 24, 1927.

In 1928, all plats were cut at or near maturity on July 7 to measure the after effects of previous cutting treatments.

TABLE IV

NITROGEN REMOVED WITH GRASSES GIVEN FREQUENT AND INFREQUENT CUTTINGS OF TOP GROWTH. GRASSES SOWN JULY 14, 1925

GRASS	FERTILIZATION	POUNDS PER ACRE OF OVEN-DRIED GRASS AND OF ELEMENTAL NITROGEN IN GRASS								
		1926			1927			1928		
		No. CUTS	YIELD	N	No. CUTS	YIELD	N	No. CUTS	YIELD	N
Blue grass (<i>Poa pratensis</i> L.)	None	1	5050	58	1	4656	55	1	1133	13
		4	6969	199	7	2093	57	1	764	12
	Abundant	1	4867	72	1	6424	80	1	4369	68
		4	7773	193	7	3571	111	1	2804	61
Fescue (<i>Festuca rubra fallax</i>)	None	1	5916	101	1	2322	20	1	888	8
		4	5316	140	7	1559	35	1	978	7
	Abundant	1	5895	77	1	3335	45	1	3021	34
		4	6030	175	7	2844	93	1	2155	31
Timothy (<i>Phleum pratensis</i> L.)	None	1	7119	87	1	4148	39	1	1849	14
		4	4576	115	7	1172	30	1	1598	14
	Abundant	1	6369	70	1	5429	79	1	7078	62
		4	4288	107	7	1981	65	1	5319	59
Red top (<i>Agrostis alba</i> L.)	None	1	7396	116	1	3016	26	1	1250	11
		4	5917	165	7	1667	45	1	1505	15
	Abundant	1	6773	78	1	5647	54	1	5016	57
		4	6587	201	7	2862	98	1	3789	61

RESULTS.—The data in table IV are presented primarily to emphasize the important differences in the amounts of nitrogen removed with various cutting treatments of the grasses in relation to their reserve content. GRABER, NELSON, LEUKEL and ALBERT (8) state that with alfalfa “nearly twice the amount of total nitrogen was removed in the top growth cut six times at succulent stages as in two cuttings taken at the seed stage.” This, of course, occurred with alfalfa which prior to cutting was well stored with reserve foods. With grasses rather similar results have obtained particularly in 1926 when, prior to cutting, the reserves in all grasses were abundant and to a less extent in subsequent years when, with frequent cuttings, there were sharp declines in yields. The percentage of nitrogen in the grasses used in this experiment, roughly may vary from three to four per cent. in early succulent stages of growth to less than two per cent. at or near maturity. When the organic reserve of grasses are sufficient to maintain abundant productivity with frequent cuttings—as was true in 1926—the removals of such immature growth make for a very heavy draft on the available nitrogen supplies in the soil so that nitrogen itself may become the limiting factor in subsequent growth. However, when such deficiencies are avoided by abundant fertility or by heavy fertilization, the available carbohydrates are rapidly reduced and may become the limiting factors of growth under a drastic cutting or grazing treatment. This is well illustrated by the yields of 1928 where all the grasses were cut but once at or near maturity on July 7. With every grass which was fertilized the yields are decidedly less when they had been cut frequently in 1926 and 1927. Whether or not frequent cuttings may for a time result in a heavier draft on other elements of soil fertility was not ascertained in these trials, but has been established by many investigators.

ORR (22) finds “a seasonal variation in the mineral content of grasses. In Scotland they tend to be richest in July and become poorer with the advance of the season. This appears to be due not so much to the season *per se*, as to the stage of growth of the plant. At the period when it is growing most vigorously and when there is a maximum of leaf full of active protoplasm, the mineral content is at its highest. If by repeated cutting the plants are prevented from attaining maturity with the accompanying increase in fibrous material in the stems and reserve organic material in the seed, the period of high mineral content is prolonged.” Similar results are indicated by HOMPER and NESBIT (10) from studies of the composition of prairie grasses in North Dakota.

While the nitrogen content of the grasses of this experiment (table IV) was not affected greatly by fertilization in 1926 probably due to an abundance of available nitrogen in the soil, the percentage of nitrogen in the

grasses in 1927 and 1928 were, with very few exceptions, much higher where nitrogenous fertilizers were applied. Such increases occurred at mature and immature stages of growth. Cutting treatments also had a subsequent effect on the nitrogen content of the grasses. In every case except one, the percentage of nitrogen was considerably higher on July 7, 1928, in the grasses cut frequently in 1926 and 1927 than in those which had been cut at or near maturity in the two previous years. This indicates a carbohydrate-nitrogen ratio, which was more favorable for vegetative growth than it was for the production of seed. There were external indications of this in the appearance of the plats of all grasses in 1929 and 1930, but this was particularly true of fescue, blue grass and timothy.

Ecological observations.—That “low” reserves in grasses resulting from frequent cutting and low nitrogen supplies in the soil tend to lower the competitive efficiency of grass and thus favor the entrance of foreign plants, especially those with a decumbent habit of growth was made evident by the general prevalence of scattered plants of white clover (*Trifolium repens*) in all the “low” reserve and unfertilized grasses (particularly fescue and red top) during 1928. Heavy nitrogen fertilization, however, practically eliminated the infestation of this clover. Timothy, low in reserve, was also abundantly infested during the summer of 1927 with thyme-leaved spurge (*Euphorbia maculata*), a decumbent plant which largely escaped mowing, but at times added considerably to the yields. The absence of all types of foreign growth was conspicuous in the plants of grasses with “high” reserves and abundant fertility.

Observations made on June 28, 1929, show again how effectively heavy nitrogenous fertilization prevented the invasion of white clover in both the “high” and “low” reserve grasses. With the absence of fertilization the plats of grasses “low” in reserves were abundantly infested with this clover, but it was much less prevalent in the unfertilized plats of high reserve grasses. Owing to the abundance of this legume in the unfertilized plats during 1929 the collection of data on yields was abandoned.

Growth recovery.—As was true with the blue grass in experiment I, all the grasses used in experiment II were slow to recover after cutting at or near maturity and particularly where such grass had been heavily fertilized. The grasses on both the unfertilized and fertilized plats which had been cut at frequent intervals in 1926 and 1927, regenerated new growth with greater uniformity and thickness after cutting on July 7, 1928, than grasses cut only at or near maturity. With the latter a tufted type of turf was particularly evident in the plats of fescue and red top, and to a much less extent in timothy and blue grass.

A tufted growth in grasses is due largely to short rhizomes and other forms of stem growth which radiate outward and which have a tendency for

annual increments in numbers and space occupied. When such grasses are allowed to mature or approach maturity before cutting it appears as if the number of individual plants which survive competition with each other is much less than when the aerial competition is reduced by cuttings. This is true if such cuttings are at a level and within limits of frequency and duration which do not greatly imperil the existence of these plants by exhaustion of reserve foods and the ingress of weeds. With fewer plants per unit area the stand is compensated for in thickness by the more rapid and abundant growth from such plants after recovery has occurred. As previously explained, however, recovery in growth after cutting at maturity may be delayed considerably due to the etiolation and death of many basal shoots and leaves occasioned by the density of growth previous to cutting. To the contrary, when top growth is retarded by ordinary deficiencies in reserves, fertility or moisture, injury to the active basal leaves and buds from the growth above is lessened so that recovery after cutting may proceed much more uniformly even though less abundantly as far as ultimate productivity is concerned. Such conditions give the impression that frequent cuttings tend to "thicken the turf" of grasses. If such thickness is measured by the number of individual shoots or leaves per unit area and by the rate and uniformity of recovery after cutting (important factors in the management of lawns and golf courses) the contention may hold, but if this statement includes the concept of productivity as measured by the total amount of top growth produced in an extended period, it may be very erroneous.

Top growth and root development of grasses and legumes grown in greenhouse under various systems of defoliation

The efforts which were made to determine the relative amounts of subterranean growth of the several grasses grown under field conditions in experiments I and II were seriously complicated by the great difficulty of separating live rhizomes and fibrous roots from those which were dead and also from partially decomposed leaves which constituted a considerable part of the turf. To obviate these obstacles, cultures of various grasses and legumes were grown in pots in the greenhouse. This made possible certain degrees of control with reference to the external environment which eliminated many field complications occasioned by drought, the ingress of weeds, insect injury, etc. When food reserves of plants are lowered by cutting treatments of the top growth, the losses in yield under field conditions which occur subsequently, may be due not only to the limitations of growth occasioned by the deficiencies of organic foods in the plants, but to many indirect influences such as a greater susceptibility to drought and winter

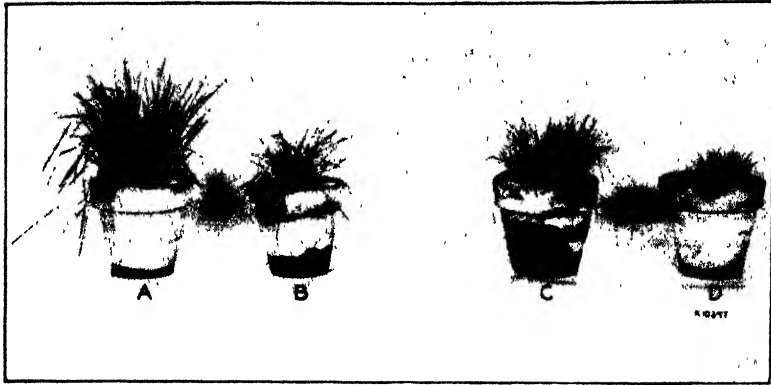


FIG. 4. Growth of red top and fescue grasses after frequent and infrequent cuttings. The grasses in pots B (red top) and D (fescue) were clipped six times between September 28 and November 13, 1928, while those in A (red top) and C (fescue) were clipped only once on November 13, 1928. The above photo was taken on December 1, 1928, after 17 days of growth in greenhouse.

injury, a lessened absorptive capacity for nutrients necessary for growth, a decrease in nitrogen supply in the soil and the invasions of weeds and other foreign growths. These complications can be avoided in greenhouse cultures so that the specific influence of the organic supply on subterranean and top growth can be more adequately measured. This was attempted in experiments III, IV and V. See also fig. 4.

EXPERIMENT III

On June 26, 1928, eight five-inch pots were filled with fertile silt loam soil. Four of these were sown with 0.6 gram each of blue grass seed, and four with 0.4 gram each of red top seed. On the same day 16 seeds of timothy were sown equidistantly in each of 9 eight-inch pots filled with similar soil. The pots were watered uniformly, and after 24 days of growth in a greenhouse, a clipping treatment was begun on 5 pots of timothy, 2 of red top and 2 of blue grass. The clippings were made one inch above the soil surface and in case of blue grass consisted entirely of leaf growth. The clippings of timothy and red top were for the most part of leaves but included some stem tissue. Seven such clippings were made on the following dates: July 20 and 25, August 2, 6, 11, 18, and 21, 1928. The grasses in the remaining pots were allowed to grow until August 24, 1928. On this date all plants were removed from pots with running water. The roots were separated from the tops. The dry weights of roots from clipped and unclipped plants at the close of a period of growth of 59 days from date of seeding are given in table V.

TABLE V

EFFECT OF CLIPPING LEAF BLADES OF SEEDLING PLANTS OF BLUE GRASS, RED TOP AND TIMOTHY ON THE AMOUNT OF ROOT GROWTH. PLANTS GROWN IN GREENHOUSE FROM SEED SOWN IN POTS OF FERTILE SILT LOAM SOIL ON JUNE 26, 1928.

DRY WEIGHTS OF ROOT GROWTH DETERMINED ON AUGUST 24, 1928.

TOTAL PERIOD OF GROWTH 59 DAYS

KIND OF GRASS	TREATMENT OF LEAF GROWTH	NO. OF POTS	NO. OF PLANTS	DRY WEIGHT IN GRAMS OF ROOTS OF 100 PLANTS
Blue grass (<i>Poa pratensis</i>).....	Not clipped ..	2	293	0.64
	Clipped 7 times	2	336	0.08
Red top (<i>Agrostis alba</i>)	Not clipped . .	2	434	1.82
	Clipped 7 times	2	393	0.10
Timothy (<i>Phleum pratense</i>)	Not clipped	4	44	7.89
	Clipped 7 times	5	69	0.55

Since nearly all the organic substances of the subterranean parts are first synthesized in the tops, a larger root growth from the unclipped plants was to be expected, but the increase from 737 to 1720 per cent. is much more than was anticipated. It is significant to add that the 293 blue grass plants not clipped before they were removed from the pots produced in addition to their root growth, 75 underground lateral rhizomes weighing 0.13 gram. The plants of clipped blue grass produced no rhizomes.

EXPERIMENT IV

Thirty pots (five inch) were filled with fertile silt loam soil which had been sifted to remove all coarse particles in order to facilitate later removal of fibrous root growth of the sweet clover, alfalfa, alsike clover, red clover, white clover, red top, blue grass and fescue which were sown broadcast and separately in such pots on August 28, 1928. The seedling plants of grasses and legumes were allowed to grow in the greenhouse for a month when clippings of the leaves (not stems) from the plants growing in one-half of the pots were made on September 28, October 4 and 13, 1928. On October 24, 1928, all the plants, clipped and unclipped, were carefully removed from the pots with running water, and the roots separated from the tops. Dry weights of the total top and root growth during a period of 57 days is given in table VI. The plants were watered uniformly by sub-irrigation, the pots being kept in water tight wooden flats. Nitrogen in the form of ammonium sulphate was added to the water applied so as to maintain a vigorous growth.

The pronounced decrease evident in root growth of all plants with three clippings of the foliar parts makes a detailed discussion of the data in table

TABLE VI

EFFECT OF CLIPPING LEAF GROWTH OF SEEDLING PLANTS OF RED CLOVER, SWEET CLOVER, ALFALFA, ALSIKE CLOVER, WHITE CLOVER, RED TOP, BLUE GRASS AND FESCUE ON THE AMOUNT OF TOP AND ROOT GROWTH DURING A PERIOD OF 57 DAYS OF GROWTH IN GREENHOUSE FROM DATE OF SEEDING ON AUGUST 28, 1928, TO DATE OF REMOVAL OF PLANTS FROM POTS ON OCTOBER 24, 1928

PLANT	NUM- BER OF POTS	NUM- BER OF PLANTS	NUMBER OF CLIPPINGS PRIOR TO OCT. 24	GRAMS OF TOTAL DRY ROOT GROWTH PER 100 PLANTS ON OCT. 24, 1928	GRAMS OF TOTAL DRY TOP GROWTH PER 100 PLANTS, INCLUDING THAT PRESENT ON OCT. 24, 1928
Red top	2	46	0	8.65	4.83
	2	62	3	2.17	0.33
Fescue	2	68	0	6.15	1.18
	2	59	3	1.58	0.25
Blue grass	2	66	0	3.61	0.72
	2	29	3	1.44	0.24
Sweet clover	1	8	0	35.13	3.92
	1	11	3	5.63	0.64
Alfalfa	2	24	0	13.46	3.00
	2	21	3	6.57	0.68
Alsike clover	2	39	0	10.20	1.65
	2	15	3	3.29	0.39
White clover	2	53	0	10.09	1.77
	2	31	3	2.37	0.28
Red clover	2	46	0	11.00	2.31
	2	36	3	3.38	0.27

VI unnecessary. With the loss of photosynthetic area and the utilization of reserves in the regeneration of new growth following the three removals of leaves, the amount of top growth produced with 57 days of growth following seeding was also very much less for the clipped plants.

EXPERIMENT V

Plants of blue grass, fescue and timothy were established in eight-inch earthen pots (six-inch pots in case of fescue) filled with a mixture of quartz and sifted fertile loam soil. The seed was sown on November 1, 1928. The number of plants which became established in each pot under the conditions of greenhouse culture varied from two to nine although in 76 per cent. of the pots the number of plants ranged from four to six inclusive. The pots were kept in water tight wooden flats which made possible uniform

applications of water to each plant as well as uniform amounts of potassium nitrate and ammonium phosphate which were added from time to time in solution so as to provide for optimum growth.

All the plants grew for 81 days, when on January 20, 1929, the top growth was cut one inch above the soil surface. On this date the plants were well established, but with no external evidence of inflorescences, except in the case of timothy where several spikes appeared. The older leaves of fescue had made a healthy dark green growth of from 4 to 6 inches in length, those of blue grass 6 to 9 inches while the older timothy stems had grown from 8 to 12 inches. The top growth of the plants in four pots of fescue, three of blue grass and four of timothy were cut again in a similar manner on January 30, February 5, 14, and 23. On the last date a second cutting of top growth was taken from the plants in the remaining three pots of fescue, three of blue grass and four of timothy. The five cuttings on part of the plants and the two cuttings on the remainder will be referred to hereafter as pre-cuts. Such cuttings were dried and weighed and the data are recorded in table VII.

On March 7 and March 12, 1929, clippings were again made on the grasses in all pots after which they were allowed to grow for a period of 43 days before the last cutting was taken on April 24. These cuttings were dried and weighed and will be referred to as recovery-cuts. On April 24, neither fescue nor blue grass had produced flower stalks while a few flowering spikes of timothy were present. The amount of top growth separated from the roots on April 24 is indicated in table VII. It consisted almost entirely of leaves in the case of fescue and blue grass, but of stems and leaves in the case of timothy.

On the day of the last cutting (April 24) the plants were removed from the pots and washed. The roots were separated from the top growth, and from the rhizomes in the case of blue grass, while with fescue the very short rhizomes were regarded as top growth and the fibrous root growth only was separated. Timothy roots were separated at the base of the stems.

The total period of growth for all plants from November 1, 1928, the date of seeding, to April 24, 1929, the date of the last cutting of top growth, and the separation of roots from stems was 175 days. During the first 81 days, the plants were unmolested; during the second 34 days, the pre-cuts were made, and during the last 60 days, the three recovery-cuts were taken.

Even though the grasses were well established with a root development of 81 days of uninterrupted growth before cutting treatments were begun, five pre-cuts of top growth yielded only one-third to one-half the amount of dry matter obtained from two pre-cuts. The five pre-cuts were taken (table VII) on January 20 and 30, February 5, 14 and 23, 1929. The two pre-cuts were taken at the beginning and the close of this period of 34 days.

TABLE VII
QUANTITATIVE EFFECTS OF FREQUENT AND INFREQUENT CUTTINGS OF FESCUE, BLUE GRASS AND TIMOTHY APPLIED AFTER 81 DAYS OF UNINTERRUPTED GROWTH IN GREENHOUSE. GRASSES SOWN IN POTS NOVEMBER 1, 1928

PLANT	No. OF POTS	No. OF PLANTS	DRY WEIGHT OF PRE-CUTS IN GRAMS 1929					DRY WEIGHT OF RECOVERY- CUTS IN GRAMS 1929			DRY WEIGHT OF ROOTS IN GRAMS 4/24, 1929
			1/20	1/30	2/5	2/14	2/23	Total	3/7	3/12	4/24
Fescue	4	21	1.477	0.335	0.152	0.121	0.106	2.191	0.180	0.083	9.95
	3	17	1.620				4.780	6.400	2.210	0.413	22.98
Blue grass	3	19	2.303	0.465	0.300	0.333	0.295	3.696	0.736	0.325	21.01
	3	17	2.140				6.570	8.710	3.025	0.623	30.74
Timothy	4	16	2.633	0.550	0.252	0.215	0.435	4.085	0.511	0.192	19.74
	4	16	2.382				6.417	8.799	1.554	0.592	33.02

* In addition to this root growth the 19 plants of blue grass produced 276 rhizomes weighing 3.64 grams (dry basis) and the 17 plants ** of blue grass developed 365 rhizomes weighing 6.45 grams (dry basis).

On March 7, twelve days after the last pre-cut was made, the first recovery-cut was taken on all the plants. Five days later (March 12) a second recovery-cut was taken. Both of these recovery-cuts consisted of succulent leaf growth varying in length from 1 to 2 inches. The grasses with only two pre-cuts made a growth recovery which quantitatively was several times that of the recovery-cuts on grasses with five pre-cuts. A third recovery-cut was made forty-three days later on April 24, 1929. At this time, the amount of top and root growth was much smaller with grass plants having been given five pre-cuts instead of two. The three additional pre-cuts made on January 30, February 5 and 14 greatly decreased subsequent productivity and constituted the only controlled factor of variance in the experiment. The greater number and quantity of rhizomes in case of blue grass given two pre-cuts is substantial evidence of the ready influence which cutting treatments of top growth may exert on subterranean development.

Summary

Marked quantitative responses of root, rhizome and top growth of various grasses (blue grass, red top, fescue and timothy) grown under field and greenhouse conditions are correlated with cutting treatments which affect the internal environment. Not only did the amount of subterranean growth and total weight of top growth, ultimately, tend to vary inversely with the frequency of defoliation but reduced growth sometimes occurred for several months subsequent to excessive defoliations. It is clearly evident that the productive capacity of grasses is not only dependent upon adequate supplies of available nutrients and moisture combined with favorable light and temperature conditions but also upon the food reserves of the plant.

When photosynthesis is interrupted by frequent removals of top growth the limitations of subterranean development may involve greater susceptibility to drought, lessened absorptive capacity and increased winter and insect injury.

The capacity of the plant for maintaining a surplus of organic foods is generally enhanced when its morphology or its inedibility or unpalatability or the type of grazing or cutting is such that a considerable portion of the photosynthetic area escapes removal. Such plant characteristics along with variations in the duration of periods of succulent growth explain, in part, the wide differences in responses of various agronomic plants to specific removals of top growth.

Frequent and close removals of the succulent top growth of grasses having abundant reserves make for a heavy draft on the supplies of available nitrogen in the soil so that the first important factor of growth limitation

may be nitrogen deficiency. When regeneration is constantly stimulated by a fertile soil or by abundant mineral and especially nitrogenous fertilization, the carbohydrate reserves are rapidly consumed and with slight opportunity for replenishment they often become the principal factors limiting growth.

When an abundant growth of blue grass occurs with fertilization and delayed cutting, the recovery after such cutting is often slow and uneven due, in part, to etiolation and the death of many basal leaves occasioned by the dense growth above. Such delay in recovery may, under favorable climatic conditions, encourage the ingress of certain weeds and may prove significant in the management of heavily fertilized pastures or of turfs and lawns where uniformity is desired.

Organic food reserves have a significant ecological relationship, especially with reference to the flora of grasslands. Aside from the occurrence of weeds with delayed cutting, the maximum competitive efficiency of beneficial grasses, as measured by invasions of other plants among them, occurred generally, when optimum fertility was combined with those practices of cutting or grazing which maintained a productive level of reserve foods in such grasses.

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SOME REACTIONS OF THE BANANA TO PRESSURE, GRAVITY, AND DARKNESS¹

ALEXANDER F. SKUTCH

(WITH FOURTEEN FIGURES)

I. Introduction

The reactions of growth of the typical dicotyledonous plant to the directive stimuli of its environment, especially to light and gravity, are described in every elementary text-book of botany. The examples chosen to illustrate these responses are almost always plants with a terminal growing point and elongated internodes. When we try to picture the behavior, under the same conditions, of a monocotyledonous plant provided with a massive false-stem, its true stem condensed to the utmost possible degree, and its growing point buried beneath a dozen layers of thick leaf bases, we are faced with a lack of precise information. Assuming, for the moment, the same inherent physiological tendencies in the rapidly growing portions of the banana or a similar plant, that we recognize in the shoot of a mint or a composite, it is difficult to imagine how these tendencies can find expression, since the young organs are imprisoned in a massive panoply of protecting leaf-bases. What, for example, would be the behavior of a banana plant placed in a horizontal position? The following experiments were undertaken in an attempt to clear up this problem.

In an earlier paper (6) I described the structure of the false-stem of the banana, but it may be convenient to characterize it briefly here. The stem of a plant which has not yet begun to "shoot," or produce its inflorescence, is a massive rhizome, expressively termed a "bulb" by the banana grower, the diameter of which usually about equals or sometimes exceeds its height (fig. 7). On the lateral and upper surfaces of this organ the leaf-sheaths are inserted in close contact with each other. In the center of the leaf-bases is the upwardly directed terminal growing point of the rhizome, which is very low, or even sunken beneath the portions of the rhizome on either side of it. The closely-overlapping leaf-sheaths form a tall and slender false-stem, the structure of which is best explained by reference to figure 1. The young leaves formed at the growing point must perform a long journey upward through the center of this false-stem before they can finally expand. Only when the plant reaches maturity and prepares to flower does the true stem begin to elongate and push up in the midst of the leaf-sheaths. It then forms a columnar shoot, rather thick, but totally unable to support itself when deprived of the enclosing leaf-sheaths. Upon it are borne, at intervals

¹ Botanical Contribution from the Johns Hopkins University no. 110.

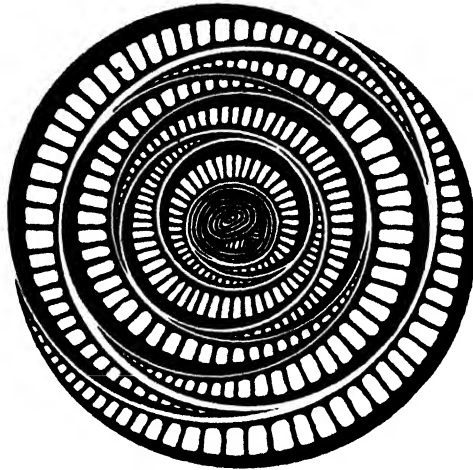


FIG. 1. Diagrammatic cross-section of false-stem. For clearness, sheaths have been separated from each other in drawing, actually they are in closest contact. Prominent lacunae occupy the greater portion of their interior. In center is lamina of emerging leaf. A large false-stem is made up of many more leaf-sheaths than are shown here.

increasingly distant, the largest leaves produced by the plant. Finally it emerges from the top of the false-stem and gives rise to the familiar pendent inflorescence. It is only with the younger plant, whose stem has not yet begun to elongate, that the present experiments deal.

II. Distribution and rate of growth

In order to understand the reactions of the banana to darkness and gravity, it is essential to know the rate and especially the distribution of elongation of the leaf sheaths. The sheath of the newly appearing lamina is in all cases elongating the most rapidly, but the sheaths of several of the older leaves surrounding this are at the same time making considerable growth. The prolonged though slight growth at the bases of the older sheaths will prove of great importance when we come to consider the geotropic erection of the false-stem.

The differentiation of the leaf of the banana is basipetal (7). When the lamina first peeps forth from the top of the false-stem (see 6, fig. 9) it is full grown. The sheath, which up to this time has lagged behind in growth, now begins to elongate rapidly. The greatest growth occurs in its basal portion, which has remained the least differentiated. The localization of growth in the base of the leaf-sheath here described rests solely upon anatomical evidence, since it has been found impossible to uncover portions of a rapidly elongating sheath, by removing segments of the older, enclosing sheaths, and mark it for growth measurements, without seriously disturbing its growth

(see section III). However, the vertical separation of the transverse septa or diaphragms (which differentiate very early) in various regions of the sheath, when compared with their separation in corresponding regions of a mature sheath, affords a ready method of determining the regions of greatest elongation. This method has proved of importance in some of the experiments which follow.

In determining the rate of elongation of the various leaves, the following method was employed. At the top of the sheath of the fifth leaf from the apex of the false-stem, a scratch was made extending from its margin on to the outer face of the next sheath within. The point of a scalpel, dipped in India ink, was found most convenient in making an enduring and easily recognized mark. The successive leaves were similarly marked, and the tip of the emerging leaf was measured in relation to a fixed point on the youngest fully expanded leaf. Thus the elongation of any leaf during a given period may be found by adding the partial increments of all the sheaths below.

The absolute rate of emergence of the leaf depends, other things being equal, upon the height of the plant, for the taller plants have longer leaf-sheaths, and these in turn possess longer growing zones. The growth of the emerging leaf is extremely rapid. MAXWELL (3) found a maximum rate of 21 cm. per day, while the writer, probably because he measured larger plants, recorded a maximum rate of elongation of the sheath of 35 cm. in 24 hours, on a vigorous plant of *Musa sapientum* subspecies *seminifera* with a false-stem about 3 m. high, growing near Almirante, Panama, during the rainy season.

Taken from the time the leaf peeps forth from the top of the false-stem, the sheath shows an increasing daily rate of elongation until a day or two before the lamina begins to unfurl (see fig. 2). Before unrolling, the lamina stands up above the false-stem like a slender rod. The process of unrolling begins at the apex, and for plants growing rapidly in a favorable environment requires from 1 to 4 days for completion, according to the size of the leaf and the vigor of the plant. During the period of unfurling, the daily rate of elongation of the sheath falls rapidly, then sinks more slowly until, when the following leaf appears, it is making 4-6 cm. growth per day (in plants 2-3 m. high under good growing conditions). The sheath continues to elongate more slowly for several weeks, and even after the second subsequent leaf has fully expanded, it usually makes 1 or 2 mm. growth per day, while a very slight growth can be detected after the third subsequent leaf has expanded. To state the same facts somewhat differently, at any given time the sheath of the emerging, still furled leaf shows the maximum rate of elongation, the first leaf below elongates 1-6 cm. daily, according to the period which has elapsed since its own expansion, the second leaf below

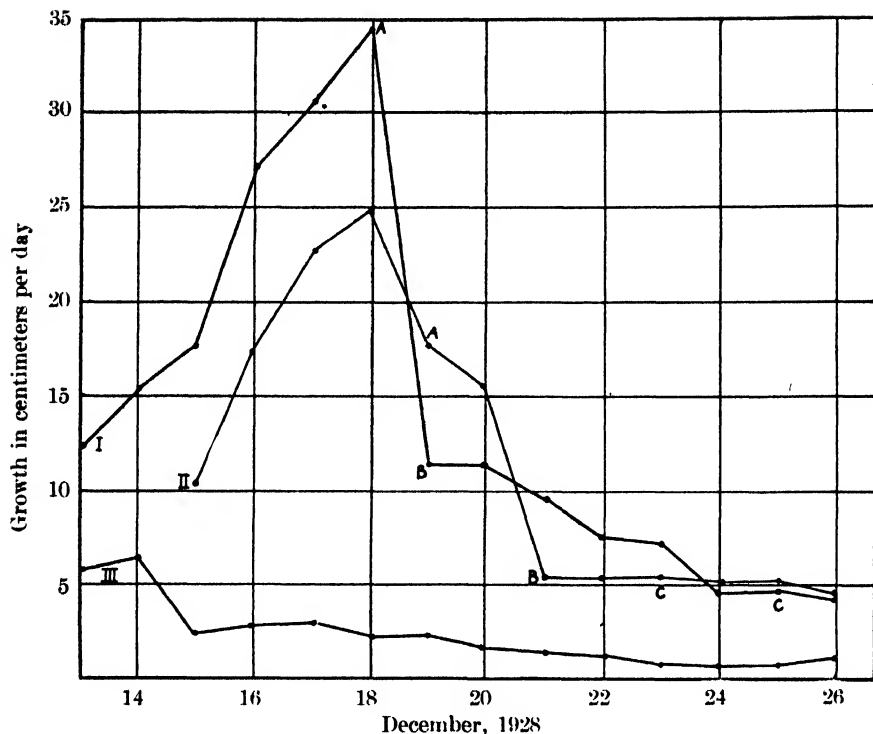


FIG. 2. Rate of growth of banana leaves. Graphs I and II give growth of two leaves from time of first appearance at top of false-stem (I on Dec. 12, II on Dec. 14) to after appearance of following leaf. A = beginning, B = completion of unfurling of lamina, C = time of appearance of next leaf. Graph III gives growth of leaf preceding I on same plant during same period. Graph I as far as C, then graph III accordingly give a fair picture of growth of leaf for a 4-week period.

elongates a few millimeters, while the third and sometimes the fourth show a slight and irregular growth. During the period from 12:30 P. M. December 17 to 12:30 P. M. December 18, 1928, the leaves of four plants grew as follows:

	Plant A	B	C	D
Emerging leaf (still furled)	170 mm.	346 mm.	349 mm.	163 mm.
Next older leaf (completely expanded)	27	22	19	13
Second older leaf	3	1	8	1
Third older leaf	1	0	3	0

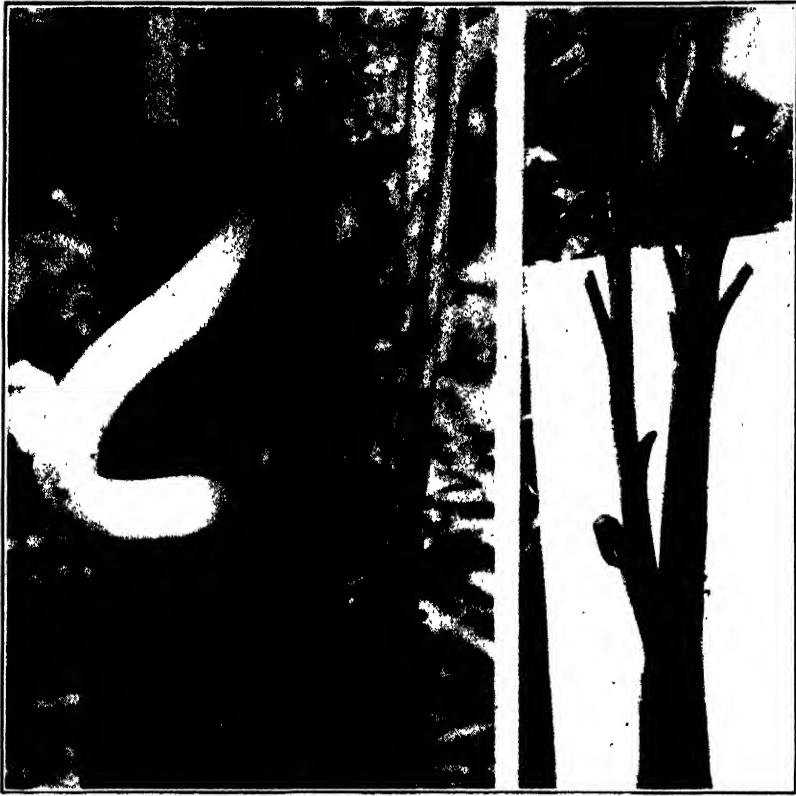
III. Pressure of growth

Great pressure is required to force the emerging leaf upward through the false-stem of a large plant. Each sheath fits tightly over those within it, and there is no wasted space. A leaf emerging from the top of a false-

stem 3 m. high is closely embraced by the sheath of the next-older leaf throughout this distance. If it is elongating at a rate of 30 cm. per day, while the sheath of the enclosing leaf is pushing upward at the rate of 5 cm. in 24 hours, then the former must slide through the latter for a distance of 25 cm. Since the growth of both sheaths is largely basal, this relative rate of motion holds good throughout most of the 3 m. stretch. The sheath of the second leaf, growing 5 cm., must slide upward through that of the third, and the third through the fourth, as long as growth continues. All surfaces of contact between the sheaths are covered with a smooth cuticula, which eases the sliding motion.

The work which the leaf must perform in emerging from the false-stem is still further increased by the necessity to spread apart all of the older sheaths as it pushes upward, because it expands gradually toward the base. Its action is that of a gently tapering entering wedge or cone. The expansion of the false-stem by the emerging leaf necessitates the lateral movement of both faces of each older sheath over all adjoining faces. In addition, these sheaths must experience a considerable change in the shape of their cross-section as they are pushed outward (compare the outer with the inner sheaths of fig. 1). The sheaths remain to a certain extent plastic and capable of slow growth-adjustments for a long time after they have ceased growth in length. The gradual maturation of the lignified mechanical elements of their tissues, which are not completely developed until the sheath comes to lie at the surface of the false-stem, as a result of the dying and shedding of those exterior to it, is significant in this connection (6, pp. 351-352).

If one cuts out a length of false-stem of say 30 cm. or more and tries to push or pull forward the portion of the emerging leaf included in it, he will find all of his strength of little avail in budging it. Its movement is possible only as a result of the slow, uniform adjustment of all the component parts of the false-stem to the growth pressures prevailing in it. The whole dynamic system is most delicately adjusted and is easily upset. The sheaths of the emerging leaves of four large plants were exposed by the removal of a rectangular section from all of the sheaths exterior to them, at a point somewhat below mid-height of the false stem (fig. 3). The operation was performed carefully, and the sheath of the emerging leaf was not injured. The cavities were filled with moist absorbent cotton, and wrapped to prevent the drying of the exposed surfaces. The experiments were performed during a rainy period, and the plants showed no indications of wilting as a result of the loss of a portion of their conducting system. The length of sheath thus exposed was 13-20 cm., the plants having false-stems 2.1-2.6 m. high. In no case did the leaf with a portion of the sheath exposed complete its emergence, although that with only 13



FIGS. 3-4. FIG. 3 (left) effect of exposing sheath of emerging leaf (see text, p. 77). Operation performed April 3, photographed April 5. FIG. 4 (right) same plant on June 4, showing production of forked false-stem.

cm. exposed pushed upward 60 cm. after the operation. In all of the others, the exposed length of the sheath began to bulge into the cavity formed in the side of the false-stem (fig. 3). The sheath, devoid at this stage of lignified elements, but consisting almost entirely of delicate thin-walled cells and large lacunae, was not sufficiently rigid to transmit the thrust of the growing base to the more apical regions of the leaf. The subsequent leaves burst through the top of the loop thus formed, and eventually a plant with a forked false-stem was produced (fig. 4), a phenomenon which I have never seen under natural conditions. Occasionally, however, one finds an inflorescence which has burst through the side of the false-stem, instead of emerging from the top, because some abnormal condition in the upper portion of the false-stem prevented its appearance in the usual manner.

The pressure, under which the tissues in the basal portion of the sheath of an emerging leaf are laboring, may be demonstrated in another way. Three large plants, with false-stems between 3 and 4 m. high, were marked for growth measurements as described above, and the growth determined at 30 minute intervals for 3 hours. The false-stem was then severed cleanly in the middle by a long, sharp knife. Almost instantaneously the cut end of the most rapidly growing sheath sprang up a few millimeters above the surface of the stump, a result of the release of the pressure against which it was growing. The total elongation of the emerging leaf was now determined at 15-minute intervals by adding (1) the emergence above the cut surface of the stump, (2) the emergence from the cut end of the top of the plant, (3) the emergence from the top of the false-stem in the normal manner. The portion included in the severed apical half of the plant was now pushing out at both ends, despite the fact that it was dis severed from its water supply, but the basal half of course made the greatest growth.

	Rate of growth during 3 hours before operation	Rate during first 15 minutes after operation	Increase in rate
Plant 1	14.7 mm. per hour	72 mm. per hour	4.9 times
Plant 2	12.7 " " "	84 " " "	6.6 "
Plant 3	12.0 " " "	108 " " "	9.0 "

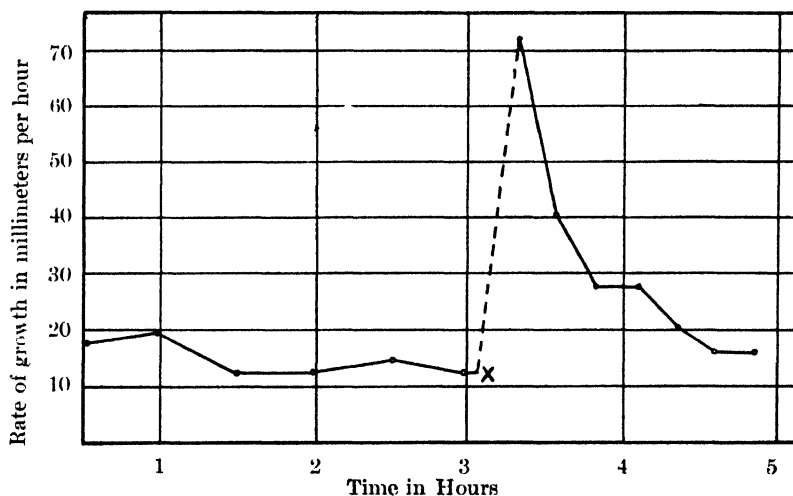


FIG. 5. Effect of relieving pressure on sheath of emerging leaf by cutting off top of false-stem on its rate of growth. X marks the point when the false-stem was severed.

After the first fifteen minutes the growth rate gradually fell, but only after 2-3 hours did it decrease to its value previous to the operation (see fig. 5).

IV. Reactions to gravity

ERECTION OF SEEDLINGS

Vigorous young seedlings readily execute geotropic curvatures. On March 9, at 8 A. M., a gardener's flat containing several dozen seedlings of the abacá (*Musa textilis* Née) was set on edge, so that the plants were horizontal. The majority of the plants were 5–7 cm. in total height, and had produced 6–7 plumular leaves. By the afternoon of March 11, they had



FIG. 6. Erection of seedling abacá (*Musa textilis*) in 56 hours after being placed horizontally.

bent upward until their false-stems formed angles of 80° – 90° with the horizontal. The curvature had taken place at the base of the false-stem, and was produced by the growth of the leaf-sheaths (see fig. 6).

ERECTION OF OLDER PLANTS

Banana plants of seedling origin when overturned preserve their ability to erect themselves from the base for a surprisingly long period, and the

largest plants tested had not yet lost this capacity. In the spring of 1929, a number of hybrid plants grown from seed, with false-stems ranging up to 2 m. in height, were available for experimental purposes. They were rooted in the ground, and were too large to be successfully transplanted to pots or boxes. Accordingly the soil on one side was gently loosened with a pitchfork, and the plants were slowly tilted over until they made an angle of 15° – 45° with the ground, when the earth was again packed around the roots. The plants suffered little injury from this treatment, and remained fresh and turgid.

In the first experiment 19 plants, with false-stems ranging from 50 to 150 cm. in height, bent upward through 20° – 50° in one week. In the second experiment, on April 8, 22 plants ranging up to 202 cm. in height were tilted over in the same manner, until they made angles of 20° – 45° with the ground. During the first week most of them curved upward through 20° – 45° , so that the most advanced now inclined only 5° – 15° from the vertical. A number were now dug up for anatomical study. The basal portion of the plant was cut in half longitudinally along the plane of curvature, and the actual curvature presented by the section was measured as accurately as possible by the use of two rulers and a protractor (see table I). The plants which were allowed to remain in the ground were in a few weeks practically erect again, and continued to grow normally.

The largest (but not the tallest) plant used in these experiments had a false-stem 189 cm. high, and 20 cm. in diameter at the base (see fig. 8 and table I, plant 10). The total height to the tip of the leaves was 385 cm.,

TABLE I

EFFECTS OF SETTING BANANA PLANTS IN AN INCLINED POSITION
INCLINED ON APRIL 8TH, DUG UP AND MEASURED APRIL 16–18TH

No.	HEIGHT OF FALSE- STEM	TOTAL CURVA- TURE	CURVATURE OF FALSE- STEM	CURVATURE ASCRIBED TO RHIZOME	DIAMETER OF RHIZOME	THICKNESS OF CORTEX	
						LOWER SIDE	UPPER SIDE
	cm.				cm.	cm.	cm.
1	156	35°	22°	13°	13.0	3.1	2.1
2	93	45	30	15	7.5	1.8	1.2
3	145	35	27	8	9.0	2.5	1.4
4	202	34	17	17	15.3	4.0	2.7
5	190	37	23	14	11.5	2.6	2.1
6	131	39	27	12	7.1	1.8	1.0
7	126	40	28	12	7.4	2.1	1.2
8	118	30	14	16	6.6	1.8	1.4
9	89	25	15	10	5.5	1.4	1.2
10	189	30			20.0	4.4	3.7

and the largest leaf possessed a blade 173 cm. long. In 8 days, this plant changed its angle of inclination through 30° , from 45° to 75° . The weight of the aerial portions (false-stem and laminae) was 15 kilograms, and the center of gravity of the leafy stem was 92 cm. above the bulb. Unfortu-

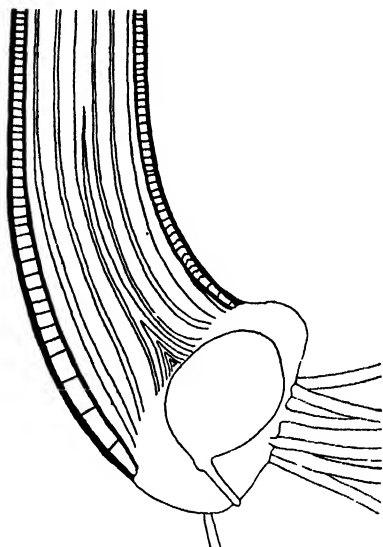


FIG. 7. Longitudinal section through base of false-stem and rhizome of banana hybrid (seedling origin) inclined at angle of 15° with horizontal March 6, dug up April 6, after it had curved upward about 70° . Note greater thickness of false-stem and cortex of rhizome on lower side. ($\times \frac{1}{2}$.)

nately the system is too complex to allow the computation of the pressure on different portions of the bulk from these data, but some idea of the magnitude of the work performed in erecting this stem may be gained from the observation that I was not strong enough to support the false stem at its original angle of inclination, when holding it at its base.

The mechanism of this geotropic curvature is complex; two distinct factors are involved in it: (1) the curvature of the bases of the leaf-sheaths and (2) the enlargement of the lower side of the rhizome, especially its cortex. While the participation of these two distinct regions in producing the total curvature is perfectly clear, the part played by each separately is in practice extremely difficult to determine. By measuring the bases of plants sectioned longitudinally, such as that represented in figure 7, I endeavored to determine the degree of curvature to be attributed to each region. The difficulties of measuring these two values separately are very great, and the values recorded in columns 4 and 5 of table I are presented



FIG. 8. Banana plant inclined at angle of 45° on April 8, photographed April 16, after it had bent upward 30° . Note curvature at base of false-stem.

with diffidence. The results indicate that from $\frac{1}{4}$ to $\frac{1}{2}$ of the total upward movement is a result of the activity of the "bulb."

Confining our attention first to the rhizome, it is found that certain very definite anatomical changes are induced there as a result of placing the plants in the inclined position. The cortex becomes very much thicker on the lower than on the upper side (see table I). This is brought about by the enlargement and division of the large, thin-walled, starch-laden mature cells of the ground parenchyma on this side. In the outer cortex, they elongate most strongly in the direction parallel to the surface (and to the long axis of the plant) and divide by one or more transverse walls. In the inner region of the cortex, the direction of elongation is not so regular, the long axes of the cells becoming often more or less strongly oblique, and the positions of the cross-walls are correspondingly irregular. Even a

month after the plants were turned over, the partition walls formed subsequent to the operation were quite distinct, since the original cell wall was still clearly recognizable, and the internal division walls were thinner and more delicate. Compared with the cortex on the lower side, that on the upper side was composed of smaller cells with walls very noticeably thicker, which were not strongly elongated in any particular direction and, except in rare cases, showed no evidence of the recent division of the mature cell. The exhaustion of starch on the lower side of the rhizome was very striking.

TABLE II

INCREASE IN THICKNESS OF LOWER SIDE OF FALSE-STEM PLACED IN
INCLINED POSITION

PLANT NO.	THICKNESS, LOWER SIDE	THICKNESS, UPPER SIDE
	<i>cm.</i>	<i>cm.</i>
11	4.2	3.2
12	8.4	7.1
13	2.4	2.5
14	3.4	3.0
15	3.9	3.3
16	4.1	3.4
17	3.5	2.7
18	3.3	2.9
19	3.1	2.6

The starch grains here were few in number and much smaller than those in corresponding regions of the upper side. WARNER (9) found an increase in reducing sugars on the lower side of horizontally placed shoots of *Helianthus annuus* and *Silphium perfoliatum*. No tests for sugars were made in the banana rhizomes, but it is probable that the starch was converted into sugar, a portion of which at least was utilized in performing the work of raising false stems.

In the region of curvature, the false-stem was distinctly thicker on the lower (convex) than on the upper (concave) side (see table II and fig. 7). The increase in thickness of the leaf-sheaths lying on the lower side is a result of the enlargement, without division, of their component cells. Since the ground parenchyma was already very thin-walled, no decrease in the thickness of the walls of these cells could be observed. The disappearance of starch grains from the cells in the leaf-sheaths on the lower side of the false-stem in the region of curvature was very pronounced. In sheaths laterally situated, it was found that the lower flank was far poorer in starch than the upper flank of the same sheath. It is of interest that the grains in the "starch-sheaths" surrounding the vascular bundles showed no ten-

dency to diminish, even when the starch was practically exhausted from the other cells in the same region of the sheath. Evidently these persistent grains serve as statoliths.

The geotropic erection of the false-stem is not brought about merely by the agency of the sheaths of the two or three inner, actively growing leaves. That these leaf-sheaths are capable of exerting great pressure, at least under certain circumstances, will be made evident in the next section, but it is difficult to understand how, with their soft tissues and the internal position which places them at a mechanical disadvantage, they would be able to exert the pressure necessary to erect the large plants in question. The one or two outermost sheaths on the lower side, the laminae of which are dying or dead, are sometimes split neatly across the thick basal portion, giving a vivid sense of the great forces displayed in the upward curvature of the false-stem. At other times, they may slip over the inner sheaths without becoming torn. But aside from these, all of the sheaths on the lower side (their number varies with the age of the plant) take an active part in the curvature. This is indicated by several circumstances. (1) The great separation of the transverse septa in the base of these sheaths shows that they have elongated in this region. (Compare the interval between these septa in the sheaths on the upper and lower sides of the plant in figure 7). (2) When the false-stem is cut across in this region, the outer sheaths on the lower side elongate, indicating that they were in a state of compression, and not passively stretched. (3) These sheaths are thicker than corresponding sheaths on the upper side, and are decidedly poorer in starch grains, evidence that active changes have taken place in them.

In the region of curvature, the fibres, though thick-walled in the outer sheaths, are generally not lignified. The hypodermal cells likewise do not become lignified at this level. Thus the stretching of the older sheaths is more comprehensible.

BÜCHER (1) described under the term geotropism the anatomical changes which occur in an orthotropous, herbaceous shoot, still capable of growth, when placed in a horizontal position and its erection forcibly prevented. Under these conditions, the cells of the collenchyma, bast and wood on the lower side acquire greater diameters than in corresponding portions of a normal shoot, while their walls remain notably thinner, at the same time these cells on the upper side show a reduced diameter accompanied by the thickening of their walls to a degree considerably above the normal. The whole cortex on the lower side becomes abnormally enlarged. These changes, which have been observed in a large number of dicotyledonous hypocotyls and epicotyls, appear to be quite general, but apparently are not appreciable in shoots which are free to bend upward under the influence of gravity. SCHWARTZ (5) has recently given a comprehen-

sive review of the literature on this subject. The increase in the size of the parenchyma cells of the sheaths and cortex on the lower side of inclined banana plants is clearly a related phenomenon. The great weight of the plants themselves causes the increase in cell size without the prevention of curvature by any external restraint.

ERECTION OF TRANSPLANTED SUCKERS

When the buds situated on the side of the old rhizome, below the insertion of the living sheaths, begin to elongate, they at first grow through the soil in a horizontal direction. At this period they produce leaves consisting of bladeless sheaths, which gradually merge into leaves with reduced, linear laminae and thick, prominent sheaths. When the young sucker reaches a certain length, which varies considerably in different varieties, it begins to bend upward. The erection of the sucker is initiated by the leaf-sheaths, which curve upward with considerable force, raising and cracking the soil above them. The rhizome of the sucker more gradually follows the young false-stem in assuming an upward direction of growth, and enlarges into the "bulb" of the new plant.

One of the methods sometimes employed in the propagation of bananas is to sever the sword suckers from the parent rhizome and, after trimming away the laminae of the leaves, to set the bulb into the ground. If such propagating material is allowed to lie in the field for some time before planting, in wet weather it usually continues to grow slowly. Occasionally one finds, in a sucker which has been lying for several weeks in a horizontal position, that the heart leaves, in their efforts to grow in a vertical direction, have burst through the side of the old false-stem and begun a new false-stem at right angles to it.

In order to study this behavior more thoroughly, on February 14, 1929, fifteen young suckers, with false-stems between 30–100 cm. high, were dug with care, so as not to injure the false-stem. These suckers had already formed massive bulbs of their own (fig. 10). All of the visible lateral buds were cut away, in order to restrict the new top-growth of the suckers to their terminal growing point. The suckers were set in the ground inclined at angles of 10° – 50° with the horizontal. Since all of the roots and none of the leaves were cut away, growth was effectively prevented until a new root system could be established. The object of the procedure was to retard the geotropic erection of the false-stem, so that when the young leaf rudiments resumed active growth, they would find themselves imprisoned within an inclined false-stem composed of sheaths which had lost the capacity of upward curvature. During the month which followed the transplanting, most of these plants succeeded in effecting more or less upward curvature, but their growth was retarded by lack of water, and they still remained

considerably inclined. No new growth of the tops was apparent, but meanwhile a new root system had become established. On March 11, the young leaves of one of the suckers burst through the upper side of the old false-stem, just above the bulb. In freeing themselves, they ruptured five living sheaths. The two oldest sheaths of the new false-stem, which had evidently exerted the greatest pressure, had torn off their laminae in escaping, leaving them imprisoned in the upper portion of the old false-stem. The pressure which it was possible for these sheaths to exert was evidently heightened by



FIG. 9. Effect of planting sucker in inclined position under conditions which delay geotropic curvature. Sucker planted at angle of 45° on Dec. 11, 1928, photographed Jan. 30, 1929. The old false-stem is shown at right, the new, formed by the young leaves bursting through base of former, has assumed an upright position.

the long period of suspended growth they had experienced, during which their tissues became more firm than is normal for the sheaths of immature leaves. The new false-stem grew rapidly, and soon formed a thriving young plant arising from the base of the old, decaying false-stem (figs. 9 and 10).

By March 27, 12 of the 15 plants had burst through the base of the old false-stem. By April 11, 13 had burst through the base and 1 had burst through the side near the top, a not infrequent occurrence in sword suckers set erect in replacing lost plants in established plantations.

After the new, upright false-stem had been formed, the growing point formed a proliferation from the old bulb, and gradually curved into an erect position (fig. 10).

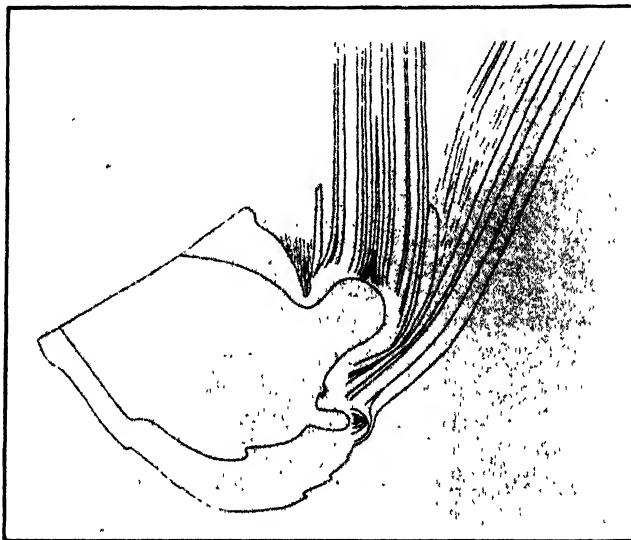


FIG. 10. Section through base of plant similar to one shown in fig. 9. Old false-stem at right; bulb severed from parent rhizome at left. ($\times \frac{1}{4}$.)

V. Reactions to darkness

REGULATION OF THE LENGTH OF THE "INTERNODES"

The regulation of the length of the leaf-sheaths is of the utmost importance to the plant. If for any reason a sheath should remain shorter than that which immediately precedes it, the lamina would not be carried free of the false-stem, could not unfurl, and the symmetry of the plant would be destroyed. In plants of banana and abacá afflicted with "bunchy top," a disease caused by a filterable virus (see OCFEMIA, 4), successive sheaths do become somewhat shorter, with the result that the leaves are crowded into an unsightly rosette at the top of the false-stem, instead of being distributed along a gracefully tapering trunk. When a sucker is transplanted without having been cut back, the leaves which would have been the next to unfold fail to emerge normally, and remain partially or completely enclosed in the false-stem, while the plant is always put to more or less inconvenience by this derangement of its growth.

It is apparent from anatomical studies that the "normal" length of the sheath is determined by inner causes at the growing point, which produces rudiments of leaves which have progressively longer sheaths, just as they

have larger laminae, until after the stem has begun to elongate rapidly and push upward through the center of the false-stem. The leaves borne on the upper portion of the fruiting stem are separated by long internodes and possess sheaths which fall off rapidly in length, since the point of their insertion is much nearer the top of the false-stem, and it is not necessary that they be so long. The laminae of these leaves also decrease rapidly in size (see 6, table I).

We call the columnar trunk of a banana plant a false-stem. By analogy, we may term the region where each petiole separates from the false-stem a "false-node," and the interval between successive false-nodes may be called "false-internodes." The false-node is not a very definite point, for the upper extremity of the sheath only gradually narrows into a petiole, and it is impossible to set a distinct boundary between the two. Accordingly, in determining the length of the false-internodes, it is best to measure from the point where the descending petiole first touches the false-stem. Since, in the young plants, the sheaths are inserted almost on a level on the abbreviated rhizome, the length of the false-internode is largely an expression of the difference between the lengths of successive sheaths. Thus, if an internode becomes longer than normal, it means that a sheath, for some reason or other, has become abnormally lengthened. Changes in the length of the false-internodes are the best measure of the influence of any condition on the length of the leaf-sheaths.

Various attempts were made to control the final length attained by the sheaths, for although limits are set to this length by the formative processes which apparently terminate long before the leaf makes its appearance at the top of the false-stem, it seemed probable that the degree of elongation of the organ could be altered, to a certain extent, by experimental means. Cutting off the top of the false-stem, so that the leaf emerges and expands sooner than normal, considerably decreases the length of the sheath, probably as a result of the earlier exposure of the lamina to light and transpiration. Cutting off the precursory appendage when it first appears from the top of the false-stem was found to have no effect on the length of the sheath. Binding the lamina with cord, as it emerged from the top of the false-stem, to prevent its unrolling, likewise had no appreciable effect on the length of the sheath. The idea that the expansion of the lamina might be the signal for the gradual cessation of elongation (see section II) therefore proved erroneous. The removal of the lamina bit by bit as it emerged likewise had no significant effect on the length of its sheath.

One's casual experience on the plantation suggests that the intensity of light is of some influence in determining the length of the false-internodes. In young suckers growing up in the shade of dense, unpruned banana groves, these internodes are very long; in shoots arising from propagating

material set out in the open, and therefore exposed to full sunlight, they are very short. Since these two types of shoots differ greatly in other important morphological respects, the conclusion that light was the controlling factor in this case could not be accepted without experimental confirmation. There is also great variation in the normal length of the false-internodes in different varieties of *Musa sapientum*. Thus, plants of the "Martini" type (subspecies *seminifera*) normally have longer false-internodes than the edible "Lady Finger" banana.

Preliminary experiments showed that young banana suckers growing in continuous darkness produce false-internodes of surprisingly great length. In one experiment a young sucker 153 cm. high produced a false-internode 43 cm. in length, while in normal suckers of the same height and variety growing in the same field, the upper internodes were 10-20 cm. in length.

Four portable houses of the type shown in figure 11 were used to study the behavior of banana plants in total darkness. Built of 1 inch boards and covered with a corrugated iron roof, they measured 90 cm. square by 210 cm. high at the front (3 x 3 x 7 ft.). Each was equipped with two venti-



Fig. 11. Three of the dark houses used in experiments, set up in field.

lators, one at the top and one at the bottom, consisting of rectangular boxes open at the ends and crossed by a series of baffle plates which, when blackened on the inside, completely excluded the light but allowed the free passage of air. They were without floors, and the door occupied almost the entire width of the front, to allow them to be easily slipped over the growing plants. The houses were lined with black paper so as to be com-

pletely light-proof, and during the course of the experiments were draped with white sheets to prevent the interior becoming too warm in the sunlight. The highest temperature recorded in them on sunny afternoons was 35° C. They were equipped with handles and shifted about the field as desired by a crew of six men.

The experiments were all performed with various undescribed varieties of the subspecies *seminifera*. Although introduced to Panama from the Philippine Islands under different native names, all showed great similarity in external characters. To start an experiment, I selected a mat (i.e., a clump of plants derived from the branching of a single individual) which contained two young suckers, already producing broad leaves, of very nearly the same height, and if possible with the two youngest visible leaves in approximately the same stage of development. The suckers were 80 cm. or less high, to allow plenty of head room in the dark chamber, and close enough together to go in easily through the door. These conditions were not always easy to fulfil, and many mats were examined before a satisfactory one was found. Except for these two suckers, all the other plants in the mat were pruned away at the ground level, and their growing points destroyed. Thus the experimental plants had the benefit of the entire root system and the large food reserves of the parent rhizome (see fig. 12). All of the laminae were then cut away from one member of the pair, while the other was left intact (see fig. 13). The plants were measured and marked so that further growth could be determined; the dark house was then moved over them, banked with earth to exclude light at the bottom, draped with sheets, and the experiment begun. Thereafter it was opened only at night, when the portion of the lamina of the defoliated plant which had emerged during the last 24 hours was cut away.¹

The duration of the experiments was limited by the height of the houses. In every case, however, the plants remained in darkness long enough for the complete expansion of at least one leaf beyond that in the process of emerging from the false-stem at the beginning of the experiment. In most cases time was allowed for two and in many instances three leaves to perform the entire process of emergence in total darkness. Thus, in every plant at least one new false-internode was added to the false-stem during the period in darkness. The more important data collected in these experiments are summarized in table III. Here both plants of each pair bear the

¹ The objection might be raised that the removal of the false-stem and growing point of the parent plant causes the suckers to produce longer internodes, just as it causes them to produce broader laminae. This, however, is not the case with plants growing in the light. In a few experiments, suckers still attached to the uninjured parent stems were enclosed in lightproof cylinders constructed of wire and black paper, and these behaved in all respects like the plants described in the text.

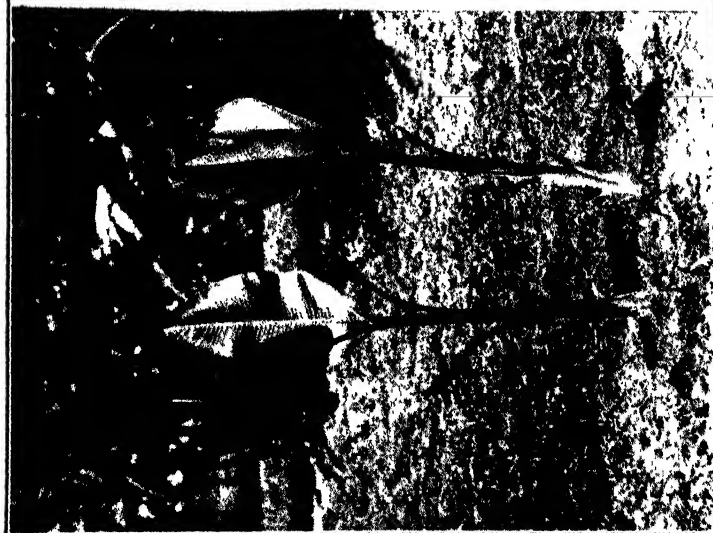


FIG. 12. A pair of plants prepared for an experiment (with the exception of removing laminae from one), showing type of suckers used.



FIG. 13. Plants 3, A and B (see table III), at conclusion of the experiment. The 12 leaves bear the white tags. April 15, 1929.

TABLE III

GROWTH OF BANANA SUCKERS IN DARKNESS

(EXPERIMENTS EXTENDED FROM MARCH 22 TO MAY 30, 1929, ONLY FOUR NUMBERS COULD BE PERFORMED SIMULTANEOUSLY)

No.	PERIOD	ORIGINAL HEIGHT		FINAL HEIGHT		GROWTH		LENGTH OF FALSE INTERNODES											
								-2			-1			+1			+2		
		A ¹	B ²	A	B	A	B	A	B	A	A	B	A	A	B	A	A	B	B
	days	cm.	cm.	cm.	cm.	cm.	cm.	cm.	cm.	cm.	cm.	cm.	cm.	cm.	cm.	cm.	cm.	cm.	cm.
1	24	70	70	125	120	55	50	14	11.5	24	17	34	23.5	0	8				
2	19	78	73	142	112	64	39	17	13	20	18	32	10	19	14				
3	24	60	65	115	93	55	28	15	14.5	19.5	12.5	33	14.5	0	0				
4	19	76	80	123	107	47	27	7.5	9.5	16	17	38.5	13	0	0				
5	24	37	41	130	102	93	61	16.5	20	24	17.5	26.5	18	11.5	8.5				
6	22	66	67	135	103	69	36	17.5	18	22	18	23.5	11	19	7				
7	22	60	63	128	95	68	32	11.5	12	17	9	32	11	25	13				
8	22	67	83	128	130	61	47	16.5	16.5	25	21.5	35	10	0	1				
9	28	59	65	140	116	81	51	12.5	17	19	17	30	20	29	9.5				
10	28	47	56	107	136	60	80	15.5	19.5	29.5	21.5	28.5	22	0	22.5				
11	21	78	79	135	112	57	33		17.5	16	21	32.5	22	30	18				
Average						64.5	44.	14.4	15.4	21.7	17.3	31.4	15.9						

¹ A = normal plant.² B = defoliated plant.

same number, the "normal" plant is designated by "A," the plant with the laminae removed by "B." The data for the A and B plants of each pair are arranged in alternating columns to facilitate comparison. The leaf partially emerged at the beginning of the experiment is designated by the number + 1, and the following leaves are numbered + 2, + 3, etc. The leaves completely emerged at the commencement of the experiment are designated by a minus sign before the number. Leaf - 1 is the youngest of these, - 2 the next in age, etc. The false-internodes are designated by the numbers of the leaves which terminate them.

We will consider first the data for the A plants. With a single exception, the longest false-internode produced by the plant was terminated by the lamina which was actually emerging when the plants were placed in darkness, indicating that the sheath belonging to this lamina was the first whose growth was markedly stimulated by the absence of light. The sheath of the next-older leaf—the youngest completely expanded when the experiment began—was also stimulated to increased growth as a result of the treatment. This is indicated by the abnormal length, in a number of plants, of the internodes terminated by these leaves, and by the marked increase (almost 7 cm.) of the average length of these internodes over those below them. Although the sheaths of 5 or 6 of these older leaves continued to grow at a rate inverse to their age, their final length was not greatly affected by the absence of light. In suckers of the size used in the experiments, growing in the light, a slight growth may sometimes be detected in even the seventh or eighth leaf from the top.

The length of the sheath of leaf + 2 is still more greatly exaggerated (beyond what it may have been in the light) than that of leaf + 1. This fact is masked, in the manner of presentation of the data we are now employing, by the abnormal length of internode + 1, and it must be remembered that the effects are additive in successive internodes. It was not possible, for lack of space, to allow these sheaths to approach their maximum length, but already several of them have formed internodes well above the maximum for plants growing in light. The length of the sheath of the youngest expanded leaf of each plant at the conclusion of the experiment is, within a few centimeters, the same as the final height of the false-stem as given in the fifth column of the table.

Suckers of the same variety and height as those used in the experiments, growing in the same field but under the shade of the older plants of the mat, rarely show internodes over 20 cm. in length. The maximum length recorded in 15 such plants chosen at random was 22 cm. Most of the upper internodes of such plants fall between 8–20 cm. in length. If the suckers are exposed to strong sunlight by cutting away the older plants, the false-internodes become much shorter. The shortest "+ 1" internode formed by

the experimental plants measured 23.5 cm., while the average of 31.4 cm. is almost 50 per cent. above the maximum for plants growing in the light. Just as in plants with elongated stems etiolation causes the production of lengthened internodes, so in the false-stem of the banana darkness causes the formation of greatly exaggerated false-internodes. This is a result of the abnormal lengthening of the sheaths of the leaves which emerge in the dark. No change in the configuration of the rhizome was observed in the experimental plants.

LOCATION OF THE INCREASED GROWTH CAUSED BY DARKNESS

The average separation of the transverse septa in the various regions of the leaf-sheaths of a plant growing in the open is shown graphically in figure 14 (at left), where the distance occupied by each 50 septa is marked off on a vertical line drawn in proportion to the length of the sheath. Here

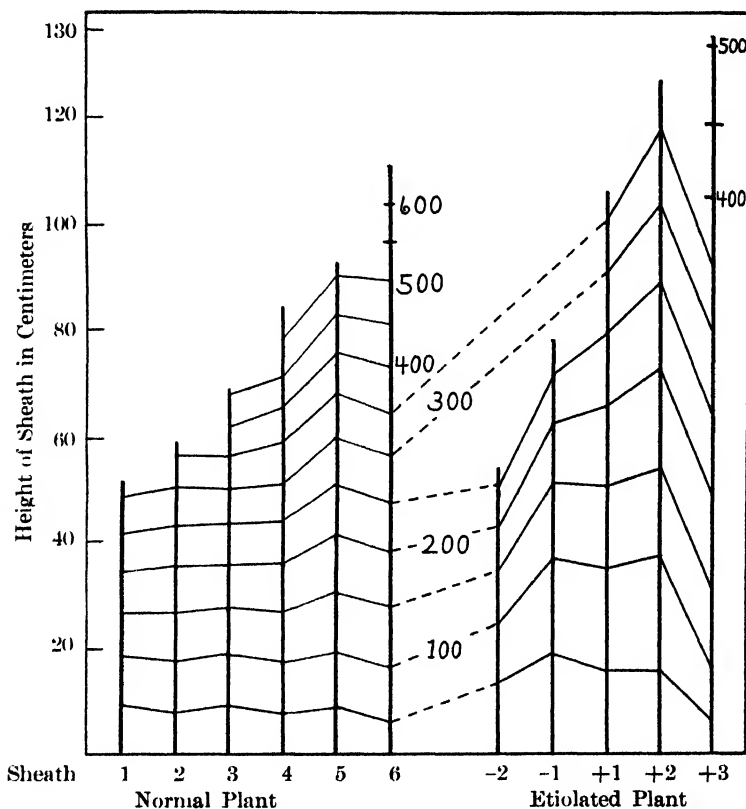


FIG. 14. Distribution of transverse septa in sheaths of normal plant (left) and plant which had been in darkness for 42 days, during which false-stem grew from 70 to 141 cm.

it will be seen that the septa are farthest apart at the base of the sheath, and gradually become more crowded toward the apex. Each figure employed in the construction of the graph represents the average of the counts of four parallel series of septa running the length of the sheath, and all from the central part. There is usually a regular increase in the number of septa in successively older sheaths. In sheath 5, however, for some reason no more septa were formed than in the preceding sheath, although the sheath itself is 9 cm. longer. As a result, the septa in all portions of sheath 5, but especially in the basal region, were somewhat more distant than in the older sheaths, indicating that the plant is able to compensate a deficiency in the formative processes by an abnormal amount of elongation at a later stage.

At the right of the figure a similar graph is presented for a plant which was kept in the dark house for 42 days, during which the height of the false-stem increased from 70 to 141 cm., and 3 new leaves (+ 1, + 2, and + 3) expanded. Leaf - 2 was affected to a certain extent by the treatment, and the increased elongation occurred chiefly in the basal portion of the sheath (first 100 septa). In leaf - 1 the increased length is accounted for principally in the region of the basal 150 septa. The separation of septa in this sheath is exaggerated by a deficiency in their number, for it contains no more than the preceding one. In the following sheath (+ 1) the separation of septa is greater than normal in all regions, but the excess growth is most marked in the basal half. In sheath + 2 there is a marked increase in the separation of the septa throughout the sheath, including its apical portions. In the sheath of the latest leaf to emerge (+ 3) the separation of the septa in the distal portion is still greater. This leaf was still rapidly elongating, and in its less mature basal portion the septa are still quite crowded.

From these data (and other similar counts which lack of space excludes here) it is evident that in leaves already expanded, or just emerging at the time the plant is placed in darkness, the increased growth is localized at the base of the sheath. In successively younger leaves the influence of darkness is felt higher and higher in the sheath, with the result that the apical regions also stretch more strongly, and their transverse septa become far more distant, than in leaves emerging in the light. It is because of the more extensive region affected in the younger leaves that they are able to maintain the exaggerated length of the false-internodes begun by the first leaves to emerge in darkness, which in each succeeding sheath means a proportionally greater stretching.

LOCALIZATION OF PERCEPTION

The intercalary growing zone at the base of the sheath of the banana leaf is covered by a number of other leaves which more or less effectively

screen it from the light. Hence it becomes of interest to determine whether the increased growth of the sheath in darkness occurs because the sheath itself fails to receive the very small amount of light it normally may receive, or as a result of changes induced by continued darkness in the lamina and thence transmitted to the growing sheath.

Observations with a diaphanoscope revealed that a certain amount of light penetrates to the base of the sheath of the emerging leaf in plants of the size employed in the experiments, although this sheath can at best enjoy a very dim illumination, most of the rays being absorbed or reflected by the 5 or 6 older sheaths through which they must filter. None-the-less, it was not *a priori* certain that the absence of this weak illumination did not cause the observed changes. To test this point, the laminae of one plant of each pair employed in the dark chamber experiments were removed as described above. The performance of these plants is recorded in table III, in the columns headed "B." Here it is seen that the plants without laminae formed internodes not longer than those commonly found in plants growing in the open. The average length of the +1 internodes of the defoliated plants is half that of the A plants, and the maximum for the defoliated plants is the same as the minimum for the normal plants. Because the false-stems of the defoliated plants remained shorter, the leaves often emerged in more rapid succession than in the normal plant of the pair, hence the earlier formation of the +2 internode observed in some cases.

In order to determine whether the removal of the laminae had in itself any effect upon the formation of the subsequent leaves or the length of their sheaths, plants growing in the open were defoliated in the same manner as the B plants in the dark houses. The newly emerging leaves of three of these plants were cut off daily for seven weeks, during which they formed 5-7 new internodes of the usual length. These plants grew in the shade of the older plants of the mat.

As a control, the false-stems of 10 suckers, most of them somewhat larger than those used in the dark houses, were wrapped in several thicknesses of heavy, black mulch paper, wound around them in a spiral like a roller bandage. By this means they were effectively darkened. The paper was covered on the outside by white cloth, to prevent its becoming heated in the sunlight. It was necessary to rewrap the stems at intervals of several days, as with increasing girth they began to press against their wrappings, and to enclose the newly formed portions. The experiment was continued 24-25 days, slightly longer than most of the dark chamber experiments. The longest internode formed by these plants measured 26.5 cm., somewhat longer than normal, but 5 cm. below the average of the +1 internodes of the A plants in the dark chamber experiments (see table IV).

TABLE IV

LENGTH OF FALSE-INTERNODES FORMED BY PLANTS WITH FALSE-STEMS DARKENED

No.	DATE BEGUN	DURATION	ORIGINAL HEIGHT	FINAL HEIGHT	LENGTH OF FALSE-INTERNODES		
					- 2	- 1	+ 1
		<i>days</i>	<i>cm.</i>	<i>cm.</i>	<i>cm.</i>	<i>cm.</i>	<i>cm.</i>
1	3-25-29	25	102	146	23.5	16	19.5
2	"	25	85	132	19.5	19	18.0
3	"	25	112	156	19.5	16	13.0
4	"	25	112	164	22.5	20.5	18.5
5	4-12-29	24	78	133	20.5	25.5	18.5
6	"	24	83	139	18.0	23.5	19.5
7	"	24	92	167	21.0	25	26.5
8	"	24	80	119	15.5	15	12.5
9	"	24	78	117	19.0	19	16.0
10	"	24	89	135	16.0	19	14.0

From these experiments it seems that the abnormal lengthening of the sheaths of plants growing in darkness is largely a result of some influence which their laminae exert upon them, although a slight direct effect of darkening on the sheaths themselves is not precluded (*cf.* table IV). This influence is transmitted to the basal growing zone of the emerging leaf, and causes an abnormal amount of elongation there, as indicated by the increased separation of the transverse septa. The influence of the leaves already in the dark chamber is likewise transmitted to the sheaths of younger leaves which have not yet made their appearance, and causes increased elongation in all portions, including regions which, at the time of the emergence of their own lamina, are too mature to be appreciably influenced directly by it.

The literature on plant hormones has already become a most extensive one, and a number of complicated phenomena of transmitted influences in the plant body seem most readily explained by the presence of such substances. WENT (10) found that a growth-promoting substance, formed in the tip of the coleoptile of *Avena* seedlings, is transmitted to the more basal portions of the organ and causes accelerated growth there. He determined that the growth-promoting substance of the coleoptile tip is formed more abundantly in darkness than in light. It is suggested that the phenomena described in this section may be caused by the production of a growth-promoting substance in the lamina of the banana leaf in darkness. This substance, carried basally in the phloem, may cause an abnormal degree of elongation of the tissues in the lower part of the sheath. It may also produce increased growth in the sheaths of younger leaves whose laminae are

still enclosed in the false-stem, and accordingly in absolute darkness are receiving only slightly less light than in a plant growing in the open. If the great distance (1 meter or more) between the organ of perception and the region of response is urged against this view, it must be remembered that the phloem in the vascular bundles in the leaf-sheath is extremely well developed (6, fig. 18) and translocation very rapid. In the fruiting banana plant, the carbohydrate formed in most of the leaves must be carried all the way down to the bulb in the leaf-sheaths, and thence all the way up the stem to the bunch of developing fruit, a total distance, in large plants of 12-14 m.

EFFECT OF ETIOLATION ON SIZE OF LAMINA

Continued darkness does not cause a reduction in the size of the lamina. The leaves which expanded in the dark chambers were in all cases longer and usually broader than those which preceded them, as is normal in immature banana plants. During the course of several of the experiments the lateral buds on the old rhizomes developed into vigorous young suckers, sometimes reaching over 1 m. in height of false-stem, and produced leaves of the dimensions usual for suckers of their size.

MACDOUGAL (2), who studied the behavior of a large number of species in darkness, found that many monocotyledonous leaves with parallel venation, arising directly from an underground tuber, bulb or rhizome, attain in darkness a length and sometimes a breadth in excess of the normal, and an area equal to or greater than the normal organ. Where there is a distinct petiole, or sheath, arising from near the ground level, these are usually excessively elongated. *Canna* agrees with *Musa* in the production of abnormally long sheaths in darkness. The laminae of many monocotyledonous leaves (especially *Araceae*) with distinct petioles or sheaths are considerably smaller than normal, and often fail to unroll completely, in this respect differing decidedly from the banana.

VI. Conclusions

A dicotyledonous plant with elongated internodes, a coleus or sunflower, for example, exhibits certain responses by which it adjusts itself when its stem is placed in a horizontal position, or its light is seriously reduced. The banana is structurally very different from these plants and although the morphological nature of the organs by which the reactions are accomplished is different, its responses to the same conditions are surprisingly similar. A sunflower reacts to darkness by increasing the length of its internodes, a banana by increasing the length of its leaf-sheaths, which results in the production of longer false-internodes. Placed on its side, the dicotyledonous plant erects itself by the upward curvature of its stem, the

banana by the upward curvature of the false-stem made up of leaf-sheaths, aided to a certain extent by the bulb. In this case the banana's method seems the more efficient, since it results in the erection of the entire false-stem from the base, not merely of the younger internodes, as is the case with the sunflower. Banana plants are able to erect themselves in this manner after they acquire a height and weight which would preclude such a response on the part of most plants. Far from being rendered relatively rigid and unresponsive by its method of enclosing the growing point within massive sheaths, the banana possesses an organization which is remarkably plastic and adjustable. It must be remembered that it is essentially a plant of quiet places; violent windstorms are disastrous to it.

In conclusion, it is a pleasure to acknowledge my gratitude to Dr. JOHN R. JOHNSTON, Director of Agricultural Research of the United Fruit Company of Boston, for extending to me the use of the Company's research stations in Central America, and to Mr. JOSEPH H. PERMAR, in charge of the station near Almirante, Panama, for his unfailing cooperation and interest, without which it would have been impossible to carry out much of the work reported here. To him I am also indebted for the communication of many valuable observations, the result of long familiarity with the banana plant. To Professor DUNCAN S. JOHNSON, of Johns Hopkins University, I owe a debt of gratitude for the interest and encouragement he has continued to show in my work.

Summary

1. Great pressure is required to force the emerging leaf of the banana upward through the false-stem. If a section is removed from the side of the false-stem, depriving the sheath of this leaf of its lateral support, it is unable to transmit the pressure exerted by the basal growing zone. The leaf accordingly is not able to emerge, and subsequent leaves break out through the aperture, forming a forked false-stem.

2. When the pressure on the basal growing zone is reduced by cutting off the top of the false-stem, the rate of elongation is immediately increased 5-9 times, but soon falls to its original value.

3. Seedlings placed in a horizontal position erect themselves by a curvature of the base of the false-stem.

4. Plants with false-stems up to 2 m. in height, 20 cm. in basal diameter, and a total weight of aerial portions up to 15 kg., erect themselves from the base when inclined at an angle of 45° or less with the ground.

5. The erection of these plants is accomplished principally by the curvature of the false-stem at its base. The sheaths on the lower side become thicker as a result of the enlargement of their cells without division. There is a decrease of starch on this side.

6. The cortical cells on the lower side of the rhizome elongate parallel to the long axis of the plant and divide by one or more transverse walls. The cell walls become thinner, and the starch grains they contained largely disappear. The whole cortex becomes thicker on the lower side than on the upper. As a result, the false-stem is pushed upward.

7. When a sucker is dug up and replanted in an inclined position, growth of the top is suspended for about a month, while a root system becomes established. Upon renewal of growth, the old false-stem is unable to erect itself. The young leaves, imprisoned within it, burst through the upper side of the old false-stem, at the base, and establish a new false-stem in an upright position.

8. When the plant is placed in darkness, the leaf-sheaths become abnormally long, with the result that false-internodes of about twice the normal length are formed.

9. Sheaths from which the laminae have been removed do not become abnormally long in the dark, although in the open the removal of the lamina does not affect the length of the sheath. When the false-stems are darkened but the laminae remain in the light, the sheaths become only slightly longer than in plants growing in the open.

10. Laminae kept in continuous darkness exert some influence on the basal growing region of their sheaths, about a meter distant, which results in their increased elongation. It seems likely that a hormone is responsible for this effect.

11. There is no reduction in the size of the lamina of plants grown in darkness.

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RELATION OF H-ION CONCENTRATION OF TISSUE FLUIDS TO THE DISTRIBUTION OF IRON IN PLANTS

R. A. INGALLS AND J. W. SHIVE

(WITH TEN FIGURES)

Although iron is an essential element for plant growth, it is not always present in an available form for assimilation by the plant. Factors influencing the availability of iron are not well understood. Previous investigations of the iron problem have dealt mainly with a study of the external medium in which the plants were grown, with less emphasis attached to the study of the conditions existing within the plants themselves.

PATTEN and MAIN (18) found that iron was precipitated from solution in varying degrees from pH 3.5 to 6.0, practically all being precipitated at 6.0 and above, thus rendering it unavailable for absorption by the plant. This was also brought out by HOPKINS and WANN (10). They found difficulty in growing plants in a medium of pH 6.0, due to the fact that iron was removed by adsorption on calcium phosphate which gradually precipitated as the solution became alkaline, a physical chemical effect capable of influencing iron availability within the plant as well as in culture media.

The fact that lack of available iron is not entirely due to the conditions existing in the culture media may be shown by reference to the work of APPEL (2). He found that buckwheat plants were less sensitive to changes in reaction of culture media than were corn plants, corn requiring much more iron than buckwheat. Little difficulty, therefore, was experienced in growing buckwheat without chlorosis in solution cultures in which corn suffered from lack of available iron. After a study of the internal conditions existing within the plant, presented in the following pages, an explanation of this phenomenon may be attempted.

LOEHWING (13) has reported that plants grown in a medium high in lime display peculiar iron immobility characterized by copious precipitation in the roots. He states that the lime reduces the sap acidity to the point of interference with internal iron mobility. Lime injury involving chlorosis has been reported for corn by MAZÉ (16), for pineapples by GILE (5), for rice by GILE and CARRERO (6), for pears by MILAD (17), and for citrus fruits by LIPMAN (22).

In addition to the work cited, some work has been reported on the H-ion concentration of tissue fluids. HAAS (8) made studies of actual and total acidities and of the total alkalinity of a number of plants of agricultural importance, together with a study of the influence of liming the soil upon

these acidities. In this connection he reports that in ten out of fourteen cases the addition of lime was followed by a decrease in actual acidity of the plant juice, which seems to point to the fact that plant juices are influenced to some degree by the nature of the medium in which they are grown. Other points of interest in his study of plant juices were as follows: (1) the presence of a hydrogen-ion concentration gradient for tissue fluids which is not always in the same direction in different species or in the stems and leaves of the same species; (2) an increase in acidity with age increase in the plant, and (3) that illumination tends to decrease the acidity of the plant. This point was further brought out by GUSTAFSON (7). He worked with *Bryophyllum calycinum*, determining the pH of its juice at various intervals throughout the day and the early part of the evening. He found that the acidity of the juice decreased during the day and increased again at night, but if the plant was kept in the dark continuously for 24 hours or more, the acidity did not continue to increase but began gradually to decrease, as the food supply was used up in respiration. The highest acidity was reached at 10 A. M. while the lowest was obtained at 4 P. M. The experiment was carried out during both clear and cloudy days, and it was found that the same general results were obtained on both types of days but to a different degree.

CLEVENGER (4) found a similar nocturnal change in pH of the tissue extract of cowpeas due to change in light intensity. He also pointed out that the pH varied with temperature, being higher at high temperatures than at low temperatures. ATKINS (3) made a study of the variation in the juices of different plants and found a range in pH from 1.4 to 8.0, but only in rare cases did he find any above 7.0.

The purpose of this investigation has been to determine first, the influence of day to night variations in light intensity upon the hydrogen-ion concentration of the plant tissue fluids of a number of different species; second, to study the relation between hydrogen-ion concentration of the plant tissue fluids, as influenced by variation in light intensity, and the "filterable" or soluble iron content of the plant tissues; third, to study the relation between hydrogen-ion concentration of the plant tissue fluids and the total iron content of the plant tissues; and finally, to study the influence of these internal factors upon the iron mobility, its distribution, and ability to function in the plant tissues.

Experimental methods

Plants used in the first part of this investigation were grown in sand and solution cultures to insure as uniform a medium of growth as possible. It was later found, however, that quite similar results were obtained with

plants grown in soil plots, provided that care was taken to select plants of the same age which had been grown under similar conditions. The species here used may be classed into two groups according to the purpose for which they were grown. Group 1 comprised buckwheat (*Fagopyrum esculentum*), clover (*Trifolium repens*), sedum (*Sedum reflexum*), and bryophyllum (*Bryophyllum calycinum*). These were grown for the express purpose of studying the influence of light intensity on the pH of the tissue fluids. The hydrogen-ion concentration of the juices of both stems and leaves of the plants of this group were determined at intervals of two hours during the day and night. Care was taken to select the various experimental periods at times when the sky was cloudless during the day in order that the plants might be subjected to the maximum variation in light intensity during a day and night period.

Group 2 comprised buckwheat (*Fagopyrum esculentum*), bryophyllum (*Bryophyllum calycinum*), rumex (*Rumex patientia*), sedum (*Sedum reflexum*), tobacco (*Nicotiana rustica*) tomato (*Lycopersicum esculentum*), asparagus (*Asparagus officinalis*), soybean (*Glycine max*), and clover (*Trifolium repens*), and were grown for the purpose of determining the filterable or soluble iron content of tissue fluids of plants having different pH values, and also to determine the filterable iron content of the tissue fluids of plants of the same species at various intervals throughout a day and night period.

Sand cultures were grown in clay percolators similar to those described by ALLISON AND SHIVE (1). They were approximately 31 cm. in height with a diameter of 15 cm. at the top and 5 cm. at the base. The opening through the bottom of each percolator was closed with a cork carrying a short glass tube through which the percolating solution could escape. The upper end of the tube was closed by a small plug of glass wool. Each percolator contained about 5 kg. of quartz sand which had been thoroughly washed in an agate pan until the overflowing water was free from sediment. TOTTINGHAM'S (25) solution modified by JONES and SHIVE (11) was supplied to each culture. This solution was continuously renewed by means of a drip and drain system (22) which allowed one liter of new solution to flow into each culture at a constant rate during a period of 24 hours. Each culture was flushed once a week with distilled water to avoid any excessive salt concentration which might have resulted from evaporation at the surface of the sand, or from water loss through transpiration. Buckwheat and soybeans were grown in solution cultures in two-quart colorless glass jars. They also were supplied with the constant solution renewal system described by SHIVE and STAHL (22). In both sand and solution

cultures, iron was supplied to the plants in the form of a freshly prepared ferrous sulphate solution containing 0.1 mg. of iron per cc. of solution.

In the early stages of growth, 0.1 cc. of the iron solution containing 0.1 mg. of iron per cc. was added to 1000 cc. of nutrient solution. This amount, however, was increased and sometimes reduced during the later stages of growth, as the external conditions and the requirements of the plants made this necessary.

Seeds used in these experiments were germinated between moist filter-papers and then transferred to a germinating net as described by SHIVE (21) When the seedlings were 4 cm. tall they were carefully selected for uniformity of size and vigor and transferred to their respective media, ten plants being used in each sand culture and three in each solution culture.

To prepare the material for the extraction of the tissue, it was cut into small pieces and placed in test tubes. These were then plugged with paraffined cork stoppers plunged into a salt-ice mixture and frozen as quickly as possible in order to prevent any appreciable chemical change before freezing. In all cases duplicate samples were used. Preparatory to expressing the tissue fluids, the test tubes containing the samples were placed in tepid water and the material allowed to thaw. This usually required from five to ten minutes. At this stage, the material was removed from the tubes and the juice extracted by means of a small screw press. Precautions were taken to prevent the tissue fluids from coming in contact with anything except glass surfaces.

pH determinations were made electrometrically by means of the hydrogen electrode and a type K Leeds and Northrup potentiometer. About 5 cc. of juice were used at each determination. It was placed in a short Pyrex tube and hydrogen was allowed to bubble through until a constant potential was attained. Electrodes were cleaned and platinized before making determinations and again frequently throughout the experiments. They were also checked against a standard acetate solution of known pH value.

Samples of green plant tissues of the various species studied, from which the moisture content and total iron content of the plant were determined, were taken at the end of each experimental interval (usually at the end of two hour intervals) throughout a day and night period. For total iron determinations, the plant tissues were dried in an oven at about 85° C. for 48 hours and at 100° C.-102° C. for 24 hours. They were then ground to a powder with a pestle and mortar in order that uniform samples might be obtained. The material was then placed in a desiccator over night, after which duplicate samples of 0.1 gm. each were weighed out and placed in Pyrex test tubes. Iron analyses were made according to a method described

by WONG (26). It consisted in completely digesting the weighed sample with 1 cc. of concentrated iron-free sulphuric acid, allowing the contents of the tube to cool for about 20 seconds and then adding to it a ten per cent. solution of sodium chlorate. This solution was added carefully drop by drop and allowed to run down the side of the tube to prevent excess spattering when the solutions came in contact with one another. Boiling was then continued until white fumes appeared. The content of the tube was clear and colorless when oxidation was completed.

Potassium sulphocyanate, 5 cc. per sample, was used as an indicator, and the contents made to a known volume with distilled water. The red color produced by the indicator varied in depth according to the amount of iron present. Each sample containing the unknown was compared in a Duboseq colorimeter against a standard iron solution. This solution was prepared by dissolving 0.7 gm. of recrystallized ferrous ammonium sulphate in about 50 cc. of distilled water. To this was added 20 cc. of 10 per cent. sulphuric acid and then sufficient one-tenth normal potassium permanganate solution was added to just oxidize the ferrous salt completely. It was then diluted to 1 liter. This solution contained 0.1 mg. of iron per cc.

To both the standard and the unknown iron solution to be determined was added 0.25 cc. of dilute (30 per cent.) nitric acid. It was found that the addition of this solution prevented the reduction of iron from the ferric to the ferrous form, thus preserving the color of the test and standard solutions without change for a sufficient length of time to compare with accuracy at least twelve unknowns against the same sample of the standard.

Since it was impossible to remove all the moisture by pressure from the plant tissue, some means had to be adopted by which total filterable iron could be determined on the basis of dry plant tissue. This was accomplished as follows:

The tissue for which iron determinations were to be made was cut into rather small pieces and thoroughly mixed. One sample was taken from the mixed tissue and frozen in order to extract the tissue fluid. Iron analyses were made on 1 cc. of the extracted filtered fluid according to the method previously described. The remainder of the plant tissue was weighed, dried, and the moisture content determined. From this, the amount of moisture per gram of dried plant tissue was calculated.

The quantitative iron analyses on 1 cc. samples of the filtered tissue fluid, together with the determinations of moisture and the content of solid material in the filtrates, furnished the necessary basis for the calculation of the iron per gram of dry material in the plant tissues in question.

Experimental results

INTRODUCTORY

The practice of supplying iron to certain species grown in artificial media in suitable proportions and in such a manner as to prevent the appearance of chlorosis from lack of this element and produce healthy green plants is attended with considerable difficulty. To accomplish this requires a knowledge of the internal nature of the particular species in question as well as the chemical nature of the culture media.

It has long been observed that not only do plants of different species require different amounts of iron, but also that plants of the same species require different amounts of iron from time to time, depending upon the degree of light intensity: that is, the iron requirement of the plants varies with the light intensity. However, this is much more pronounced in some species than in others. Among the species exhibiting the characteristic of marked variation in iron requirement with variation in light intensity are those in which the pH of the tissue fluids lies close to or above the precipitation point of iron; and such plants are frequently employed in experimental work. In this type of plant, chlorosis may occur under certain conditions from lack of iron in the chlorophyllous cells with an adequate concentration of available iron in the culture media. It was found that the proper concentrations of available iron required in the culture medium to prevent chlorosis in these plants during a period of low light intensity, such as a period of cloudy weather, was entirely inadequate to maintain the normal green color in the plants during periods of high light intensity, such as a series of successive clear days during the summer months.

On the other hand, those species in which the pH of the tissue fluids is considerably below the precipitation point of iron do not exhibit any marked variation in the iron requirement with variation in light intensity, nor is the iron concentration in the medium required to maintain the healthy green color in these plants ever so great as it must be for the type of plant in which the pH of the tissue fluids lies near or above the precipitation point of iron. It is thus evident that the cause of chlorosis due to the lack of iron is largely dependent upon factors existing within the plant, through the agency of which the iron may be precipitated at certain points along the paths of translocation in stems or leaves, thus preventing the migration of iron into the chlorophyllous cells.

It is well known that under most field conditions where the soil solution is slightly acid in reaction, plants can usually obtain a sufficient supply of iron during all phases of growth. In certain alkaline soils, however, some important agricultural plants are unable to obtain a sufficient amount of

available iron, thus causing considerable loss in production, while under the same conditions other plants appear to suffer no injury from lack of available iron. It is reasonable to assume, therefore, that an internal mechanism renders small quantities of iron mobile in the plant and available to the chlorophyllous cells. It was with these points in mind that the present investigation was undertaken.

The influence of light intensity on the H-ion concentration of plant tissue fluids

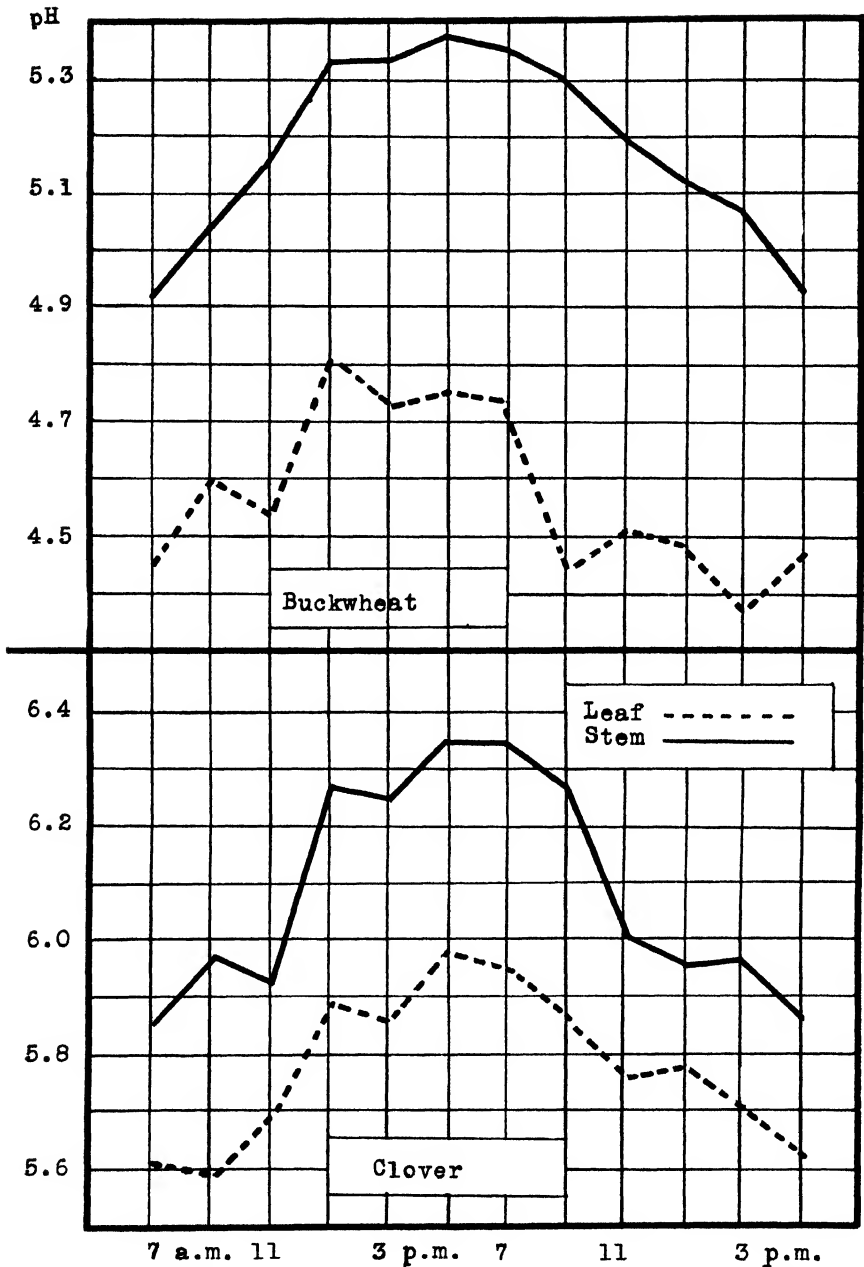
The data obtained from the experiments conducted for the purpose of showing the effect of light intensity on the hydrogen-ion concentration of plant tissue fluids are presented in tables I and II. In the first column of each table is given the time at which pH determinations of tissue fluids were made during a period of 24 hours. In the succeeding columns are given the average pH values of juices of stems and leaves of the plants indicated at the head of the respective columns. Each value represents the average of two or more determinations. The results given in table I are all from plants with thin leaves, while those of table II deal with thick-leaved, fleshy plants.

Figure 1 shows the graphs plotted from the data as given in table I, representing the course of change in the pH values of the stem and leaf juices of buckwheat plants during a period of 24 hours. The experiment from which these data were obtained was carried out in the spring of the year, the

TABLE I

pH VALUES OF TISSUE FLUIDS OF STEMS AND LEAVES OF BUCKWHEAT, CLOVER AND *Rumex* PLANTS AT TWO HOUR INTERVALS

TIME	BUCKWHEAT		CLOVER		<i>Rumex</i>
	STEMS	LEAVES	STEMS	LEAVES	LEAVES
9:00 A. M.	4.615	5.047	5.596	5.968	4.210
11:00 A. M.	4.531	5.139	5.697	5.934	4.193
1:00 P. M.	4.804	5.342	5.883	6.272	4.328
3:00 P. M.	4.726	5.333	5.868	6.238	4.480
5:00 P. M.	4.767	5.376	5.985	6.342	4.514
7:00 P. M.	4.757	5.351	5.951	6.340	4.311
9:00 P. M.	4.446	5.300	5.866	6.255	4.277
11:00 P. M.	4.548	5.190	5.765	6.002	4.176
1:00 A. M.	4.497	5.139	5.783	5.951	4.193
3:00 A. M.	4.353	5.089	5.714	5.968	4.108
5:00 A. M.	4.447	5.021	5.630	5.850	4.032
7:00 A. M.	4.454	4.920	5.613	5.850	4.057



FIGS. 1 (upper) and 2 (lower). Graphs representing the course of change in pH values of leaf and stem juices of buckwheat and clover plants during a 24-hour experimental interval.

sky being perfectly clear during the experimental interval, so that the plants were exposed to approximately maximum variation of light intensity from day to night for this period of the year. These graphs clearly bring out the fact that there is considerable change in hydrogen-ion concentration of the juices of both stems and leaves which decreases during the day with increase in light intensity and increases during the night. It will be observed that the juices of buckwheat stems are much more acid than are those of the leaves, the stems having a maximum pH of 4.79 and a minimum of 4.45, while the corresponding pH values for leaves are 5.37 and 4.92, respectively. While there is a marked difference in pH values between stems and leaves, as indicated by the graphs, the range of variation in these values over a 24 hour period is about the same for the juices of both stems and leaves. Not only are they alike in this respect, but also there is a pronounced similarity in the general trend of the graphs representing the course of change in pH values. It will be observed, however, that the graph representing pH values of stem juices is somewhat less regular in its general outline than is that representing pH values of leaf juices. Highest H-ion concentration for stems is indicated at 3 A. M. and for leaves at 5 A. M., while minimum H-ion concentrations (maximum pH value) are indicated for stem and leaf tissue fluids at 1 P. M. and 5 P. M., respectively. Although the pH of the stem juices appears to fall after 1 P. M., there is no immediate pronounced decrease until 5 P. M., when the values decrease quite rapidly following the rapid decrease in light intensity.

In figure 2 are given the data for clover as taken from table I and plotted in the same manner as are those for buckwheat in figure 1. The experiment from which these data were obtained was carried out on a clear day similar to that on which the data for buckwheat were obtained. It will be observed that the pH values for the juices of clover are much higher than for those of buckwheat, these values for the juices of clover being above the precipitation point of iron during the day and slightly below this point during the night period. Nevertheless, the range in daily variation appears to be much the same in both species. Here again, the juices of stems are much more acid than are those of the leaves, the stems having a maximum pH value of 5.98 and a minimum of pH 5.59, while the leaves have a maximum of 6.34 and a minimum of 5.83. Highest acidity is indicated at 7:00 A. M. and lowest acidity at 5:00 P. M.

The next plant to be considered is *Sedum*, which is a thick leaved, fleshy succulent. The data for this species are presented in table II and are graphically shown in figure 3. It will be observed that this plant shows the daily change in hydrogen-ion concentration to a much more marked degree than do those already considered. In stems, the range is from pH 4.78 at 7:00 A. M. to 5.49 at 7:00 P. M., while the leaves show a corresponding range

TABLE II

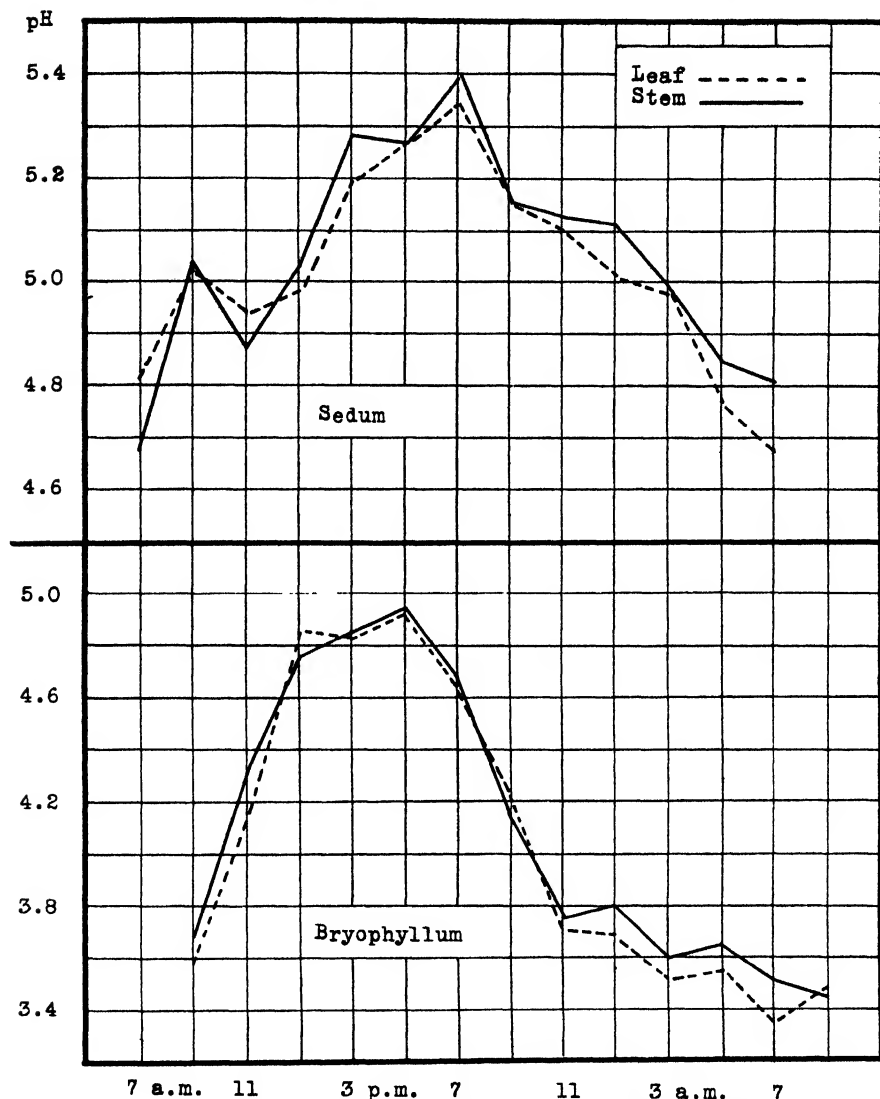
pH VALUES OF TISSUE FLUIDS OF STEMS AND LEAVES OF *Sedum*, *Bryophyllum* AND *Tradescantia* PLANTS AT TWO HOUR INTERVALS

TIME	<i>Sedum</i>		<i>Bryophyllum</i>		<i>Tradescantia</i>	
	STEMS	LEAVES	STEMS	LEAVES	STEMS	LEAVES
9:00 A. M.	5.131	5.122	3.685	3.584	4.641	4.395
11:00 A. M.	4.970	5.038	4.311	4.125	5.241	5.207
1:00 P. M.	5.122	5.081	4.750	4.852	5.528	5.453
3:00 P. M.	5.376	5.292	4.835	4.818	5.461	5.384
5:00 P. M.	5.359	5.359	4.936	4.920	5.511	5.495
7:00 P. M.	5.494	5.440	4.666	4.649	5.545	5.494
9:00 P. M.	5.249	5.249	4.159	4.243	5.148	5.173
11:00 P. M.	5.224	5.198	3.753	3.719	5.351	5.300
1:00 A. M.	5.207	5.114	3.821	3.685	5.275	5.266
3:00 A. M.	5.087	5.080	3.618	3.516	4.666	4.556
5:00 A. M.	4.869	4.945	3.652	3.550	4.548	4.717
7:00 A. M.	4.784	4.911	3.516	3.347	4.599	4.646
9:00 A. M.			3.449	3.483	4.954	4.903

from 4.91 at 7:00 A. M. to 5.46 at 5:00 P. M. In this plant the pH values of stem and leaf juices show no significant differences and the graphs representing these values follow somewhat the same course throughout. This appears to be characteristic of fleshy, thick leaved plants such as were here used.

Bryophyllum, another of the thick leaved, fleshy plants, shows an extreme range in the pH values of its tissue fluids from day to night, as is indicated by the graphs of figure 4. This range is more than double that indicated for *Sedum*. The maximum pH 4.92 for leaf juices occurred at 5:00 P. M. and the minimum pH 3.34 at 7:00 A. M., while the corresponding maximum and minimum values for stems, pH 4.93 and pH 3.44 are shown for 5:00 P. M. and 7:00 A. M., respectively. The graphs representing the courses of pH values for stems and leaves throughout the experimental period run quite closely together, but again there are no significant differences between stem and leaf values such as are indicated for the thin-leaved plants.

In table I and table II are presented also data for *Rumex* and *Tradescantia*, dealing with the influence of light intensity on the H-ion concentration of plant tissue fluid. These data are not here discussed, and are presented merely to emphasize the fact that acidity change with variation in light intensity is a phenomenon common to many types of plants and occurs



FIGS. 3 (upper) and 4 (lower). Graphs representing the course of change in pH values of leaf and stem juices of *Sedum* and *Bryophyllum* plants during a 24-hour experimental interval.

in proportion to the degree of succulency. This is further emphasized by tests of many species, the data for which are not here presented.

It has long been known that the succulent plants exhibit periodic rise and fall of the acid content of their juices, and the relation of this phenomenon to the respiratory processes has been the subject of extensive and thor-

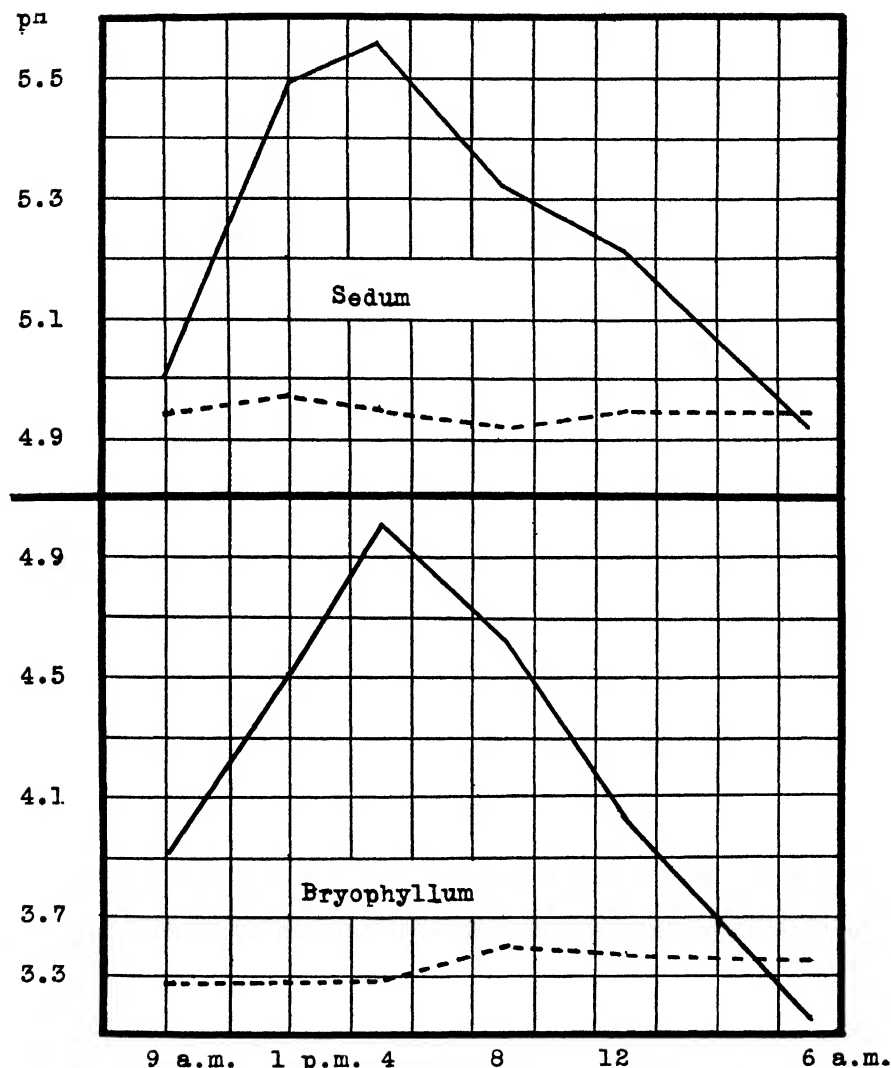
ough investigation. Both RICHARDS (19) and SPOEHR (23) have shown that the most important single factor which leads to the formation of acids in fleshy succulents is a low or insufficient oxygen supply, while their disappearance is mainly due to the photolytic action of light which breaks them down into simpler substances. But whether the low pH values of plant tissue fluids in the absence of light are the result of the formation and accumulation, under these conditions, of titratable organic acids, or whether they are due to the formation of carbonic acid from the accumulation and solution of carbon dioxide in the plant juices at night, as appears to be indicated by the work of MAQUENNE and DEMOUSSY (14) are questions which have not been experimentally investigated.

The present investigation, however, is not concerned with the causes underlying the accumulation and disappearance of organic acids in plants, but only with the fact that pH fluctuations of plant tissue fluids do occur, not only in fleshy succulents but also, to a lesser extent, in plants exhibiting a low degree of succulency, and that these fluctuations are directly related to variations in light intensity. To demonstrate that light is the important factor in the diurnal pH fluctuations of plant tissue fluids, a comparison was made of the juices of the plants of *Bryophyllum* and *Sedum*, exposed in the greenhouse to alternating day and night during 24 hour periods, with the juices of plants of the same age grown under similar conditions, but kept in dark chambers in the greenhouse during corresponding 24 hour periods. Care was taken to keep the temperatures approximately equal around the plants inside and outside of the dark chambers

TABLE III

pH VALUES OF THE TISSUE FLUIDS OF *Sedum* AND *Bryophyllum* PLANTS IN CONTINUOUS DARKNESS AS COMPARED WITH THOSE EXPOSED TO INTERMITTENT PERIODS OF LIGHT AND DARKNESS

TIME	<i>Sedum</i>		<i>Bryophyllum</i>	
	LIGHT	DARK	LIGHT	DARK
	<i>pH</i>	<i>pH</i>	<i>pH</i>	<i>pH</i>
9:00 A. M.	5.004	4.936	3.922	3.483
1:00 P. M.	5.495	4.970	4.514	3.483
4:00 P. M.	5.562	4.953	5.004	3.483
8:00 P. M.	5.325	4.920	4.632	3.615
12:00 P. M.	5.207	4.953	4.023	3.584
6:00 A. M.	4.920	4.936	3.347	3.550



FIGS. 5 (upper) and 6 (lower). Graphs representing the pH values of tissue fluids of *Sedum* and *Bryophyllum* plants which were exposed to intermittent (unbroken line) and continuous (broken line) periods of darkness.

during the experimental periods. Data for such a comparison are given in table III and are shown graphically in figures 5 and 6.

It will be observed that the juices of the plants exposed to alternate light and dark show the usual wide range in pH values, while the juices of the plants kept in continuous darkness show only very slight fluctuations which are not at all related to the light factor.

It may be of interest here to emphasize the point that comparisons of plant tissues or tissue fluids, particularly with respect to pH values and also, as will be brought out later, with respect to soluble iron content, can be of little value unless the samples upon which quantitative measurements are made are collected at the same time during the day or night. External conditions, particularly light intensity, which is subject to almost continuous fluctuation, have a pronounced influence upon these internal factors and may render any set of measurements of them useless for purposes of comparison unless careful attention is given to the collection and preparation of experimental material.

Relation of pH values to soluble iron content of plant tissue fluids

It has been suggested by HOFFER and CARR (9) that the mobility of iron and aluminum salts in plants is associated with high sap acidity, and they have shown that under certain conditions relatively large quantities of iron and aluminum will accumulate in different parts of corn plants. It has also been shown by MARSH and SHIVE (15) that plants under certain conditions may become chlorotic from lack of iron in the leaves when the total iron content of the plants is excessively high. Furthermore, in view of the fact that the iron requirement of plants is relatively high during periods of high light intensity and low during periods of diminished light, it is of interest to determine whether or not the soluble or *filterable* iron in plants bears any relation to the periodic fluctuation in the pH values of the tissue fluids resulting from variations in light intensity.

Accordingly, the soluble (filterable) iron content of tissue extracts taken at regular intervals throughout 24-hour experimental periods was determined for a number of species. The manner of taking samples, preparing the extracts, and the technique employed in making the pH tests and the chemical analyses have already been described. The data are presented in table IV. This experiment, like those previously described, was carried out on clear days so that the plants were exposed to approximately maximum variation in light intensity from day to night.

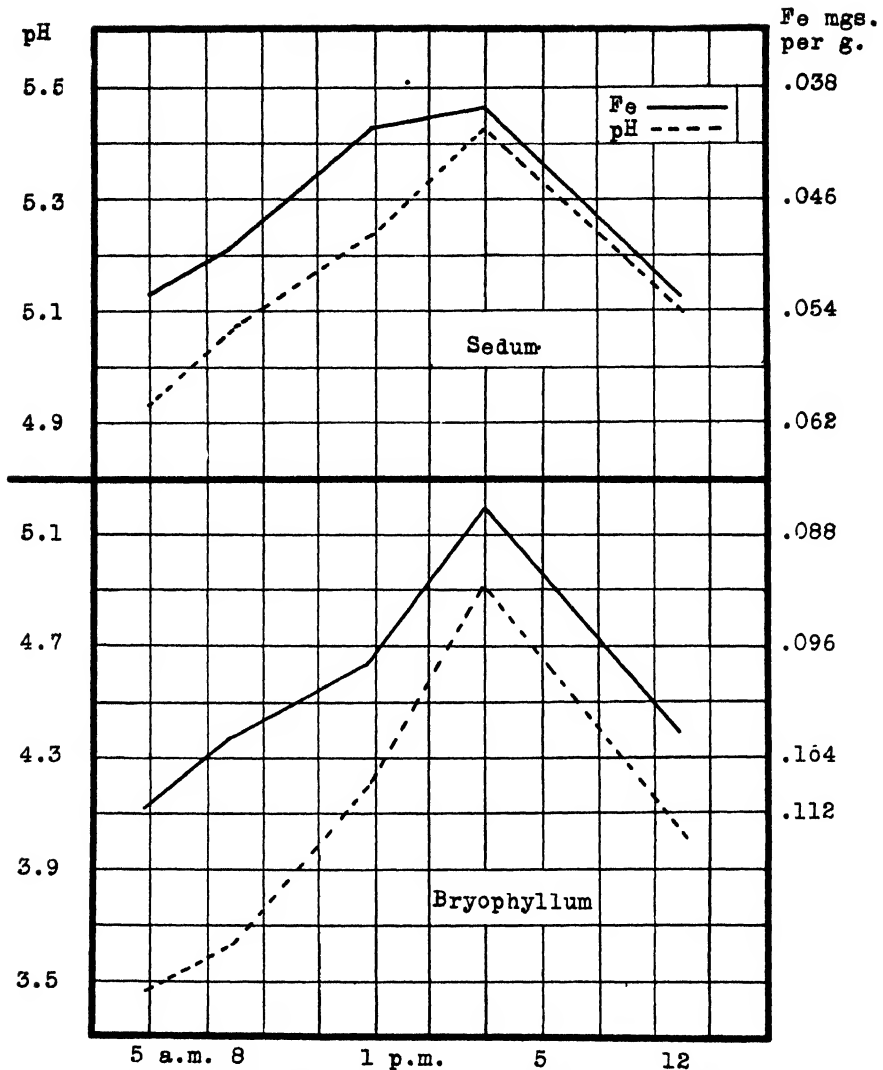
Examination of the data of table IV brings out the fact that there is a direct and very exact relation between the H-ion concentration of the tissue fluids and the soluble iron content of all the species investigated. In each species, the fluctuation in pH values of the plant juices with variation in light intensity is followed, in the inverse order, by a corresponding fluctuation in the soluble iron content. That is, for each species, low pH values correspond with high soluble iron content and high pH values with low soluble iron content.

To bring out the exactness of this relation and to show the course of fluctuation of the soluble iron content in the plants during a 24-hour period,

TABLE IV

pH VALUES AND SOLUBLE (FILTERABLE) IRON CONTENT, EXPRESSED AS MG. PER GRAM OF DRY TISSUE, AT INTERVALS DURING 24-HOUR EXPERIMENTAL PERIODS

PLANT	TIME	STEMS		LEAVES	
		ACIDITY	FE	ACIDITY	FE
		<i>pH</i>	<i>mg.</i>	<i>pH</i>	<i>mg.</i>
Asparagus	6: 00 A. M.	5.478	0.0342	5.687	0.0299
	12: 00 Noon	5.528	0.0277	5.850	0.0239
	5: 00 P. M.	5.664	0.0245	6.086	0.0223
	12: 00 Night	5.511	0.0302	5.816	0.0267
Clover	6: 00 A. M.	5.630	0.0327	5.934	0.0316
	12: 00 Noon	5.866	0.0202	6.137	0.0275
	5: 00 P. M.	5.968	0.0241	6.357	0.0247
	12: 00 Night	5.731	0.0329	5.985	0.0289
Soybean	6: 00 A. M.	5.731	0.0354	5.883	0.0330
	12: 00 Noon	5.782	0.0300	5.923	0.0299
	5: 00 P. M.	5.830	0.0247	6.230	0.0212
	12: 00 Night	5.799	0.0333	5.960	0.0244
Tobacco	6: 00 A. M.			5.579	0.0438
	12: 00 Noon			5.765	0.0315
	5: 00 P. M.			5.833	0.0364
	12: 00 Night			5.664	0.0445
Tomato	5: 00 A. M.	5.190	0.0392	5.579	0.0314
	9: 00 A. M.	5.207	0.0373	5.718	0.0299
	1: 00 A. M.	5.461	0.0331	5.985	0.0256
	5: 00 P. M.	5.562	0.0298	6.154	0.0237
	9: 00 P. M.	5.528	0.0361	6.036	0.0266
	1: 00 A. M.	5.308	0.0345	5.799	0.0295
Buckwheat	5: 00 A. M.	4.395	0.0751	4.903	0.0588
	9: 00 A. M.	4.427	0.0762	4.970	0.0566
	1: 00 P. M.	4.598	0.0668	5.038	0.0489
	5: 00 P. M.	4.804	0.0626	5.359	0.0445
	9: 00 P. M.	4.767	0.0676	5.258	0.0518
	1: 00 A. M.	4.362	0.0768	5.089	0.0588
Sedum, whole plant	5: 00 A. M.	4.936	0.0535		
	8: 00 A. M.	5.055	0.0502		
	1: 00 P. M.	5.241	0.0429		
	5: 00 P. M.	5.427	0.0399		
	12: 00 Night	5.122	0.0535		
Bryophyllum	5: 00 A. M.			3.483	0.1071
	8: 00 A. M.			3.612	
	1: 00 P. M.			4.210	0.0972
	5: 00 P. M.			4.896	0.0861
	12: 00 Night			4.023	0.1020
Dock, whole plant	5: 00 A. M.	4.091	0.0762		
	8: 00 A. M.	4.193	0.0640		
	1: 00 P. M.	4.226	0.0557		
	5: 00 P. M.	4.480	0.0513		
	12: 00 Night	4.362	0.0640		



FIGS. 7 (upper) and 8 (lower). Graphs representing the course of variation in pH values and soluble iron content of *Sedum* and *Bryophyllum* plants during a 24-hour experimental interval.

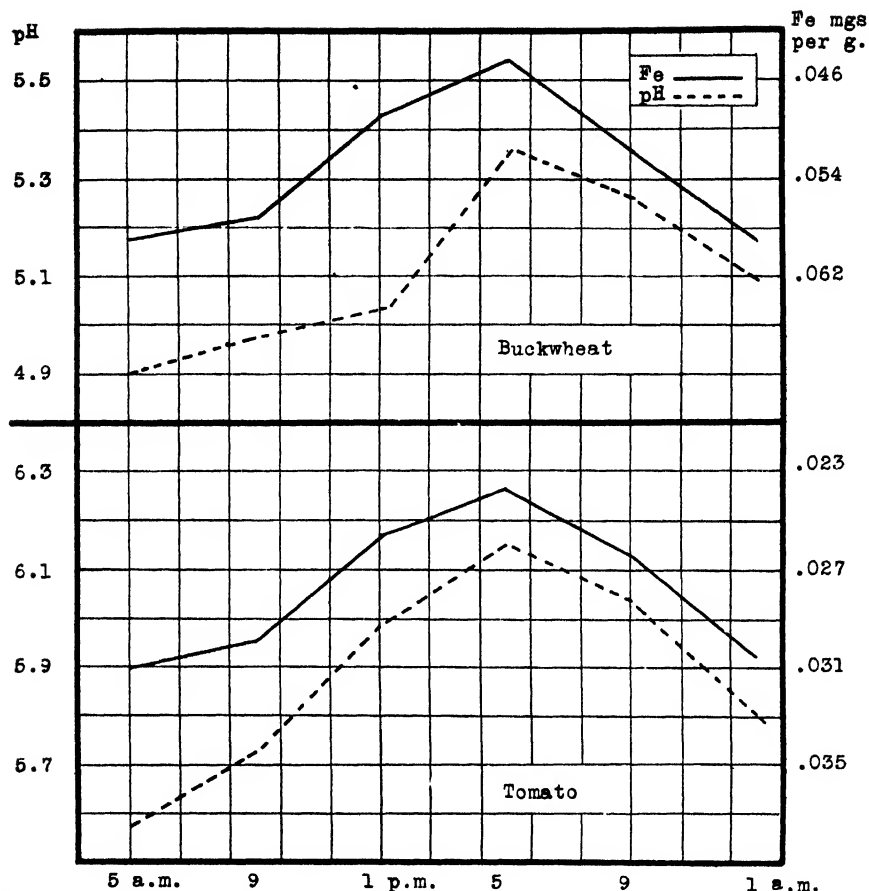
the data for two species of fleshy succulents (*Bryophyllum* and *Sedum*) and for two species of thin-leaved plants showing a relatively low degree of succulency (tomato and buckwheat) have been plotted to form the graphs of figures 7 and 8, and 9 and 10. The pH values and the values for the soluble iron content for each of these species are plotted together to form a pair of graphs with common abscissas, the ordinates on the left indicating

pH values, those on the right expressing soluble iron content (mg. per gram of dry plant tissue). To avoid intersecting of the graphs, the ordinates on the right are written in the inverted order.

The lower graph for each species (figs. 7 and 8) shows the usual course of pH change during a 24-hour period and this, in every case, is almost duplicated by the inverted graph representing the course of fluctuation in the soluble iron content during the same period, thus indicating an intimate relation between pH values of tissue fluids and that portion of iron in the plant which may be regarded as the active fraction, on the reasonable assumption that filterable iron here considered is mobile, readily available, and capable of functioning in the plant processes.

Another significant and important relation is here indicated. A comparison of the data for *Bryophyllum* with those for *Sedum* (fleshy succulents, figs. 7 and 8) brings out the fact that the juices of the former show relatively low pH values, ranging between 3.48 and 4.90, with a relatively very high content of filterable iron, ranging between 0.0861 and 0.1071 mg. per gram of dry tissue; while the juices of the latter show higher pH values, 4.94 to 5.43, with a correspondingly much lower content of filterable iron, ranging from 0.0399 to 0.0536 mg. per gram of dry tissue during a 24-hour period. A comparison of the data for tomato and buckwheat (thin-leaved plants with relatively low degree of succulency, figures 9 and 10) shows this relation in an equally definite manner. Of the four species graphically considered, the tomato shows the highest pH values, varying between 5.58 and 6.15, and the lowest content of filterable iron, fluctuating between 0.0314 and 0.0237 mg. per gram of dry tissue, during an experimental period of 24 hours. This relation holds for all the species the data for which are presented in table IV. Furthermore, the relation holds also for different organs of the same plant, as between stems and leaves, when these organs show considerable difference in the pH values of their juices. This is clearly shown by the data in table IV for stems and leaves of buckwheat, tomato, asparagus and soybeans. The significance of this relation will be further considered in the following section.

It might be well here to suggest that from the data thus far presented it appears that those plants in which the pH values of the tissue fluids lie close to the precipitation point of iron (about 6.0) show greater fluctuation in the filterable iron content from day to night than do those plants in which the pH values of the tissue fluids lie considerably below the precipitation point of iron. This is probably to be inferred from the fact, as will be brought out later, that in plants of the latter type a high percentage of the total iron is in the soluble form, and under such conditions small fluctuations in this fraction might be expected. However, this is merely put forth as



FIGS. 9 (upper) and 10 (lower). Graphs representing the course of variation in pH values and soluble iron content of buckwheat and tomato leaves during a 24-hour experimental interval.

a suggestion, since not sufficient evidence of a positive character is at hand to warrant a definite conclusion.

Comparison of soluble and total iron content of plants with pH of tissue fluids

It has already been pointed out that in certain types of plants, only a very low percentage of the total iron in the tissues is in the soluble (filterable) state, while in other types of plants nearly all the iron is in the soluble state. A study was made of a number of species showing a range in the average pH values of tissue fluids from 4.04 to 6.10, in order to emphasize the relation which pH values of the tissue fluids bear to total, insoluble, and soluble iron fractions in the various species.

TABLE V

AVERAGE pH VALUES, TOTAL AND SOLUBLE IRON CONTENT OF VARIOUS PLANTS
OVER A 24-HOUR PERIOD

PLANT	LEAF			STEM		
	FE, PER GRAM OF DRY TISSUE			FE, PER GRAM OF DRY TISSUE		
	ACIDITY	TOTAL	SOLUBLE	ACIDITY	TOTAL	SOLUBLE
	<i>pH</i>	<i>mg.</i>	<i>mg.</i>	<i>pH</i>	<i>mg.</i>	<i>mg.</i>
Bryophyllum	4.04	0.137	0.0958			
Rumex (whole plant)	4.24	0.219	0.0622			
Buckwheat	5.10	0.269	0.0516	4.58	0.082	0.0696
Sedum (whole plant)	5.16	0.278	0.0477			
Tobacco	5.74	0.325	0.0390			
Tomato	5.87	0.297	0.0277	5.38	0.089	0.0349
Asparagus	5.87	0.373	0.0256	5.54	0.111	0.0214
Soybeans	6.01	0.469	0.0246	5.79	0.142	0.0308
Clover	6.10	0.571	0.0281	5.78	0.214	0.0299

The data for the various species presented in table V are arranged in the ascending order of average pH values of the different species. The data were obtained by collecting samples of each species at regular intervals (two or four hour intervals) throughout twenty-four hour periods on clear days. The various measurements were made on these samples and the corresponding values obtained for each species were then averaged; so that each value in the table represents the average of the values obtained over a twenty-four hour period.

It will be observed that the total iron content increases in the different species as the pH value of the tissue fluids increases: that is, high total iron in any given species corresponds to high pH value of the tissue fluids and low total iron with low pH values. Thus, *Bryophyllum* with the low average pH value of 4.04, shows also a relatively low total iron content of 0.137 mg. per gram of dry tissue; while clover with a pH value of 6.10, shows the abnormally high total iron content of 0.571 mg. per gram of dry tissue. On the other hand, the soluble iron content of the different species varies in the inverse order with variation in pH values of the tissue fluids. That is, low pH values correspond to high soluble iron, and high pH values correspond with low soluble iron. Thus, *Bryophyllum* with a low average pH value of 4.04 shows a low total iron content (0.137) but relatively high soluble iron (0.0958); while clover with a pH of 6.10 shows the abnormally high total iron of 0.571 mg. per gram of dry tissue but a very low content of soluble iron (0.0281). These relations hold, not only for the different species, but

also for different organs of the same plant, as between stems and leaves when these organs show considerable difference in the pH values of their tissue fluids, as has already been pointed out.

From the foregoing considerations it is quite apparent that plants like *Bryophyllum*, *Rumex* and others with relatively low pH values of the tissue fluids, absorb only very small quantities of iron, and that practically all of the iron absorbed remains in a soluble form and is presumably available and capable of functioning in chlorophyll production and other plant processes. But plants such as clover, soybeans, and others with high pH values of the tissue fluids absorb relatively very large quantities of iron, if this is available in the external medium, but only a small proportion of this remains in a soluble form in the plant. Much of the total iron in plants like these is precipitated, probably along paths of translocation, and is therefore unavailable and undoubtedly does not function in the plant processes. If, for any reason, all of the iron in plants of this type should become soluble at any one time, iron toxicity would probably follow and might result in the death of the plant.

The cause for the accumulation of relatively large quantities of unavailable iron in the tissues of plants in which the pH values of the sap lie close to the precipitation point of iron, or the mechanism by which this is accomplished, is at present not clear. It may be suggested, however, that through the precipitation of iron in the plant tissues this element is removed from the field of osmotic activity and thereby a diffusion gradient for it may be maintained from the outside to the inside of the plant, resulting in the accumulation of relatively large quantities of non-available iron.

From the data here presented, it is to be expected that plants in which the pH values of the tissue fluids lie close to or above the precipitation point of iron may yield a high total iron content, but only a small proportion of this total iron can function in chlorophyll production and other plant activities. This is made clear, not only by chemical analyses of the tissues and tissue fluids, but also by the fact that chlorosis is likely to occur in these plants from lack of available iron under slightly unfavorable conditions, and particularly under conditions of high light intensity during periods of which the plant sap attains its maximum pH value and the plant its minimum value for soluble iron. On the other hand, plants in which the pH values of the tissue fluids lie considerably below the precipitation point of iron show relatively low total iron, but nearly all this iron can be extracted with the tissue fluid and is capable of passing through a quantitative filter-paper of the highest quality. This iron appears to be quite mobile in the plant and is uniformly distributed, as is indicated by quantitative analyses of the tissues of the various plant organs, and it is therefore rea-

sonable to assume that this iron is capable of functioning in the plant processes.

Summary

1. The hydrogen ion concentration of tissue fluids varies with light intensity: low hydrogen ion concentration corresponds to high light intensity, and high hydrogen ion concentration to low light intensity.

2. Fleshy or succulent plants show greater variation in hydrogen ion concentration of tissue fluids with change in light intensity than do thin leaved plants, the range of variation occurring in proportion to the degree of succulency of the plants.

3. All plants studied show differences in hydrogen ion concentration between leaf juices and stem juices; fleshy or succulent plants show much lower differences, however, than do non-succulent plants, the degree of difference being in proportion to the degree of succulency.

4. A comparison of hydrogen ion concentration of tissue fluids of different species has no significance whatever unless determinations are made from material collected at the same time from plants grown under approximately identical conditions.

5. Soluble (filterable) iron content of plants varies directly with the hydrogen ion concentration variation brought about by changes in light intensity from day to night.

6. Plants in which the tissue fluids have low hydrogen ion concentration values show high total and relatively low soluble iron content; and those in which the tissue fluids have high hydrogen ion concentration values show low total iron and relatively high soluble iron content.

7. In all plants studied, the iron content of leaves is higher than the iron content of stems.

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PHENOLASE ACTIVITY IN RELATION TO SEED VIABILITY

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(WITH THREE FIGURES)

Introduction

Oxidase and catalase activity of plant tissues are so frequently used as criteria of metabolism and viability that methods for their quantitative measurement are becoming increasingly important in physiological technique. Quantitative data for catalase, however, are more numerous than those of other oxidases, probably because of the difficulty encountered in making accurate determinations of oxygenases and peroxidases. There are, nevertheless, several important reasons for more frequent estimations of oxidase activity. The physiological function of catalase is still obscure. Oxidases, on the other hand, have been closely associated with the ability of living organisms to bring about transformations of materials otherwise stable at ordinary temperatures. Though our knowledge of oxidation-reduction reactions *in vivo* is as yet fragmentary, the abundant and diverse experimental evidence which has accumulated makes inescapable the conclusion that oxidases are essential to co-ordinated respiratory and other oxidative activities of plants. The correlation between oxidases and physiological processes has been so definite that these enzymes have been placed in a causal relationship to them. The desirability of a convenient quantitative method for oxidase determination is consequently self-evident.

Colorimetric methods for estimation of oxidases have been widely used. These methods have involved the use of such reagents as alpha-naphthol, hydroquinon, pyrogallol, paracresol, ortho- and para-aminophenol and others. Though solutions of these reagents are to varying degrees autoxidizable in air, the amount of spontaneous oxidation can be minimized by keeping the hydrion concentration within definite limits for each reagent (6). All of them, however, are appreciably autoxidized in nearly neutral media. This is especially true of two common reagents, phenylenediamine hydrochloride and demethyl-phenylenediamine, which have as a consequence been little used as such in colorimetric estimations of oxidases.

In an attempt to procure an understanding of enzymatic processes in plants, objection arises to any appreciable modification of the hydrion concentration of the reaction medium from that approximately normal to the plant. Realization of this fact has resulted in a search for reagents which are not greatly autoxidized in neutral media (6). A few oxidase reagents of this type, such as alpha-naphthol and paracresol are available but they do not produce the steep color gradients of more sensitive chromogens. Other

things being equal, sensitive reagents reveal more minute differences and produce sharper contrasts. These characteristics and the fact that the procedure with certain precautions can be made quantitative readily justify the use of these reagents despite the apparent complexity of the process. An experiment was undertaken with the Nadi reagent to determine its utility in the estimation of indophenol oxidase activity in certain plant tissues. This reagent has found general application in animal physiology but only infrequent use thus far in plant problems (7, 9, 10, 12). This report describes the use of the Nadi reagent in a study of seed viability.

Experimental procedure

The colorimetric procedure for determination of phenolase activity involved essentially the following steps, namely, the preparation of a completely oxidized color standard in terms of which the phenolase activity and spontaneous autoxidation are expressed, the determination of the phenolase activity of a unit quantity of seed powder, and the spontaneous autoxidation of the reagent containing a unit quantity of inactivated seed powder. The colorimetric reading of the latter was then subtracted from the reading obtained for the active enzymatic material and the result recorded as the actual phenolase activity.

The color standard was prepared by taking 125 cc. of M/100 α -phenylenediamine, an equal amount of M/100 α -naphthol, 62 cc. of 0.25 per cent. sodium carbonate and placing them in a 500-cc. bottle through which washed air was vigorously bubbled for five days to insure maximum coloration. The solution was then made up by volume to 70 per cent. alcohol. It was observed that the percentage of alcohol, acidity, and alkalinity of the solution greatly influenced the color. The resulting oxidized solution had a pH of 7.0. Since the accuracy of the colorimeter, other things remaining constant, depends upon having nearly the same color intensity in the standard and unknown, the two were always balanced as nearly as possible. Dilutions of the standard made were primarily for the purpose of increasing the accuracy of the colorimeter determinations.

A series of convenient dilutions was made by pipetting 1 cc. of the completely oxidized Nadi reagent into 40 cc. of 70 per cent. alcohol, 1 in 60, 1 in 80, 1 in 100, 1 in 150, 1 in 500, and 1 in 1000. These color standards were designated in the order given as 40, 60, 80, etc. The enzyme powder was in all cases maintained at a concentration of 1 per cent. while the intensity of the color standard employed was chosen with respect to the phenolase activity.

A small number of assorted seeds was ground in a Nixtamal mill, only seeds apparently free from mechanical injury, and infection being selected. Each group of seeds was labelled to identify it in relation to variety

and age. Lots of seeds were then analyzed at random to avoid possible error due to preconceived notions. The powder was then screened through an 80 mesh sieve. In the case of wheat a few determinations were made with coarser material which passed through the 40 mesh but not the 80 mesh screen. In all other determinations only the material which passed the 80 mesh screen was used, the object being to secure material as nearly uniform in physical texture and composition as possible. The powder, though no definite attempt was made to differentiate the various parts of the seeds, was predominantly endosperm and embryo tissue.

In determination of phenolase activity the sieved powder was weighed and made up to 1 per cent. by volume with boiled, distilled water at pH 6.8 (2, 3, 14). This solution was then allowed to stand for 90 minutes. In the meantime small quantities of dimethyl-para-phenylenediamine hydrochloride (hereafter referred to as phenylenediamine), α -naphthol, and sodium carbonate were weighed. The sodium carbonate was used to neutralize the acidity of the phenylenediamine. When the aqueous enzyme solution has stood for 90 minutes at room temperature (23° C. to 26° C.) the weighed quantities of α -naphthol and phenylenediamine were made up with distilled water to a concentration of M/100. This is approximately the concentration employed by other workers in similar investigations (5, 7, 10), and it was found to give satisfactory results with the seeds used in this experiment.

The phenylenediamine and α -naphthol solutions were not made up and mixed until used as they are relatively unstable and autoxidizable in the presence of air. The substances, however, are quite stable in the solid form. α -naphthol as used in this experiment was made up by adding equal parts of distilled water and 95 per cent. alcohol. An aqueous solution of α -naphthol was also prepared by boiling 8 gm. of the solid α -naphthol in 1 liter of distilled water for about 150 minutes. The solution was then made up to 1 liter by volume, cooled, and filtered. A few determinations were then made to see if the 9 per cent. alcohol retarded the reaction. No retardation was observed and consequently the alcoholic solution of α -naphthol was used in all of the experimental work. It was found that 1 cc. of 0.25 per cent. sodium carbonate was sufficient to neutralize the phenylenediamine, giving the final pH as 7.5 for the total substrate. A 2-cc. portion of α -naphthol, an equal amount of phenylenediamine, 1 cc. of sodium carbonate and 5 cc. of an infusion made from 1 part of seed powder in 100 parts of distilled water were then pipetted into a 9.5 cm. petri dish. The shallow petri dish was used to increase the surface area in contact with air (5). The reaction was allowed to proceed for a given period of time with occasional shaking and the solution was then made up by volume to a 70 per cent. alcohol concen-

tration. The frequency and duration of shaking were constant for each group of seeds.

It was observed that the increase in the alcoholic concentration inhibited the enzyme action but did not stop the autoxidation of the reagents. A time period of ten minutes was adequate for the resulting di-amino-compound to become uniformly dispersed in the alcohol. DYE (5) working with animal tissues found it necessary to allow 15 minutes for complete dispersion of indophenol in alcohol. A difference in the physico-chemical properties in plant and animal extracts may account for the difference in the time period required for the reaction.

A volume of the colored solution was then decanted and taken out of the petri dish and its color intensity determined in a Duboseq colorimeter against the color standard. For a control, 2 cc. of phenylenediamine and equal volume of α -naphthol, 1 cc. of sodium carbonate, and 5 cc. of enzyme solution were pipetted into a petri dish and made up by volume to 70 per cent. alcohol. This made the concentration of the control similar to that of the unknown, thus affording a fairly accurate measure of the spontaneous oxidation of the Nadi reagents in the presence of atmospheric oxygen. Temperature variations in all of the experimental work remained in the range of 22.9 to 26.3° C. The per cent. oxidation of indophenol which might be formed was divided into the actual amount formed as determined in the colorimeter, and this was expressed as percentage.

In the determination of catalase activity an apparatus similar to that employed by APPLEMAN (1) was used. The determinations were made by placing 1 gm. of the seed powder in a 250-cc. Erlenmeyer flask, with 20 cc. of hydrogen peroxide, with reaction period (ten minutes) and the shaking constant throughout. Corrections for variations in atmospheric pressure were made and the corrected gas volume was recorded.

The per cent. germination of seeds was taken by selecting multiple lots of 25 apparently perfect seeds. These were then treated for 5 minutes in 0.5 per cent. mercuric chloride solution, washed with distilled water, and then placed in sterile petri dishes containing a small amount of uniformly moistened absorbent cotton. The seeds were at all times subjected to a saturated atmosphere. A germination count was made at the end of each day for 5 days. Temperature variation during the germination period was from 25 to 34° C. All seeds remained free from infection during the germination period.

The seeds used in the experiments had been stored under ordinary laboratory conditions and although no attempt had been made to provide absolutely uniform conditions throughout the period of storage, they were not exposed to abrupt temperature or moisture fluctuations.

The experimental data are presented in the accompanying tables I-V.

TABLE I
AGE, PHENOLASE ACTIVITY, AND GERMINATION

AGE	POWDER	REACTION TEMPERATURE	OXIDATION	GERMINATION
MARQUIS WHEAT				
<i>years</i>	<i>mesh</i>	<i>°C.</i>	<i>per cent.</i>	<i>per cent.</i>
3	80	23.8	14	24
5	80	23.1	23	60
6	80	23.0	24	76
7	80	23.0	16	28
8	80	23.1	13	12
3	40	23.5	9	24
5	40	23.0	25	60
6	40	22.9	24	76
7	40	23.4	14	28
8	40	23.3	13	12
MICHIGAN AMBER WHEAT				
1	80	25.7	21.8	32
6	80	25.6	21.4	0
13	80	25.6	13.0	0
1	40	25.8	16.0	32
6	40	25.8	12.0	0
13	40	25.8	10.0	0

TABLE II
AGE, PHENOLASE ACTIVITY, AND GERMINATION

AGE	REACTION TEMPERATURE	OXIDATION	GERMINATION
PINK OF WINTER WHEAT			
<i>years</i>	<i>°C.</i>	<i>per cent.</i>	<i>per cent.</i>
1	25.1	50	64
3	25.0	29	8
4	24.3	39	32
6	24.4	18	0
1	25.1	37	64
3	25.0	33	8
4	24.2	29	32
6	24.8	26	0
SUCCESS BEARDLESS BARLEY			
1	25.9	23	64
4	25.7	22	12
11	26.1	18	8

TABLE III
AGE, PHENOLASE ACTIVITY, AND GERMINATION

AGE	REACTION TEMPERATURE	TIME		OXIDATION	GERMINATION
		REACTION	TOTAL		
SWEDISH SELECT SPRING OATS					
<i>years</i>	<i>°C.</i>	<i>min.</i>	<i>min.</i>	<i>per cent.</i>	<i>per cent.</i>
1	25.5	10	20	18.33	16
2	25.5	10	20	18.19	16
3	25.9	10	20	16.86	52
4	25.5	10	20	16.21	52
5	25.5	10	20	14.00	12
6	25.3	10	20	15.10	16
7	25.3	10	20	12.76	0
8	25.2	10	20	12.00	0
9	25.1	10	20	10.45	0
1	25.3	45	60	75.90	16
2	25.3	45	60	75.60	12
5	25.3	45	60	75.80	0
7	25.3	45	60	75.96	0

TABLE IV
AGE, PHENOLASE ACTIVITY, AND GERMINATION

AGE	TEMPERATURES		TIME		OXIDATION	GERMINATION
	INCUBATION	REACTION	REACTION	TOTAL		
ARLINGTON WHITE SPINE CUCUMBER						
<i>years</i>	<i>°C.</i>	<i>°C.</i>	<i>min.</i>	<i>min.</i>	<i>per cent.</i>	<i>per cent.</i>
2	25.4	25.4	20	40	27.0	56
3	25.4	25.4	20	40	26.8	52
4	25.4	25.4	20	40	27.2	28
2	26.7	26.1	10	20	15.0	56
3	26.1	26.1	10	20	14.0	52
4	26.1	26.1	10	20	10.0	28
2	52	26.3	10	20	13.0	56
3	52	26.3	10	20	12.0	52
4	52	26.3	10	20	8.0	28

MANCHURIA SPRING BARLEY

1	25.9	25.9	10	20	34	76
4	25.9	25.9	10	20	27	46
11	25.9	25.9	10	20	24	12
1	52	26.4	10	20	30	76
4	52	26.4	10	20	26	46
11	52	26.4	10	20	24	12

TABLE V
RELATION OF CATALASE ACTIVITY AND AGE IN SEEDS

AGE	AMOUNT	POWDER	AMOUNT OF GAS	GERMINATION
ARLINGTON WHITE SPINE CUCUMBER				
<i>years</i>	<i>gm.</i>	<i>mesh</i>	<i>cc.</i>	<i>per cent.</i>
4	0.2	40	14.55	56
4	0.4	40	26.4	56
3	0.2	40	14.8	52
3	0.4	40	32.0	52
2	0.2	40	15.1	28
2	0.4	40	33.1	28
MICHIGAN AMBER WHEAT				
1	0.4	60	34.0	32
6	0.4	60	27.5	0
13	0.4	60	17.0	0

Discussion

In view of differences in interpretation of the indophenol reaction (7) and because of the exploratory character of the present investigation no attempt has been made in this report to adopt a critical definition of the terms oxidase and phenolase. The terms are employed simply to designate the oxidizing components of certain seeds. Although there is a certain amount of doubt regarding the exact course of the Nadi reaction there is consensus of opinion about the principle involved and the nature of the end products. It seems entirely justifiable, therefore, to employ the Nadi method in testing the phenolase activity of seeds. The Nadi method has already been proved experimentally satisfactory, it is quantitative, and there is agreement concerning the fundamental character of the reaction.

Analysis of the data (tables I-V) reveals that there is no simple quantitative correlation between phenolase activity, age and germination as determined by the Nadi reaction. If, however, both the age and percentage germination are taken into consideration then a definite though not proportional relationship becomes apparent. Those samples which were taken from relatively young seeds and had a high degree of germination gave correspondingly a large oxidation percentage. The fact revealed in table I, that the three year old seeds, in the case of Marquis wheat, have a lower percentage oxidation than 5 year old seeds is explained, in part at least, by the fact that the percentage of germination of the 5 year old grain is more than twice as great as that of the 3 year old. Oxidase activity consequently was in this instance more closely correlated with viability than with age.

Similar results have been reported by CROCKER and HARRINGTON (4) for *Amaranthus retroflexus*. Examination of the experimental results obtained from the 7 year old wheat with a germination of 28 per cent. as contrasted to 8 year old grain with 12 per cent. germination shows a decrease in oxidation as the concomitant of age and low vitality.

A difference in the amount of oxidation with variation in size of the mesh through which the seed powder has passed was also noted (table I). The powder which passed through the 40 but not the 80 mesh sieve gave more irregular results for both Marquis and Michigan amber wheat. While the percentage oxidation was more variable in the 40 mesh than in the 80 mesh powder it will be noted that the relationship to age and percentage germination of the seeds had the same general trend. The percentage of oxidation for the two varieties of wheat at the same age is not identical nor is there a definite correlation of Marquis and Michigan wheat in relation to germination and oxidation. It will also be noted (table I) that the 6 and 15 year old Michigan amber wheat did not germinate but that the latter gave a lower oxidation percentage. This indicates that there is a definite relation between age and phenolase activity.

The parallelism in catalase and phenolase activity in seeds of given age is of interest. The latter may prove to be the more reliable index to viability if the procedure for its determination can be standardized and simplified.

There appears to be a positive relationship between catalase and phenolase activity in Arlington white spine cucumber and Michigan amber wheat (fig. 1). The phenolase and catalase activity approximately parallel each other in relation to the age of seeds. The decrease in activity of the two enzymes accompanying senescence indicates that the intensity of the catalase

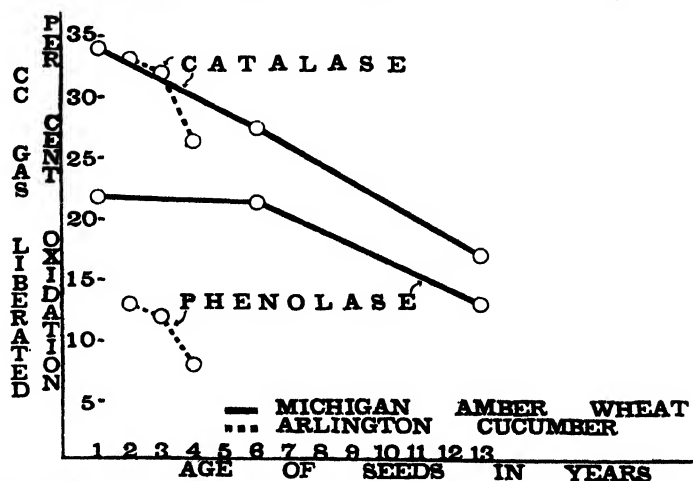


Fig. 1. Comparison of phenolase and catalase activity in seeds of wheat and cucumbers.

activity had no appreciable effect upon the amount of indophenol produced (7).

The same generalizations in relation to age and per cent. of germination apply to the Pink of Winter wheat (table II).^{*} The three year old Pink of Winter wheat with 8 per cent. germination gave a lower percentage oxidation than the four year old wheat of the same variety but with 32 per cent. germination. This situation is similar to that occurring in Marquis wheat (table I). These two cases indicate that there is a greater amount of correlation between oxidation and germination than between age and oxidation in the case of wheat. Success beardless barley, on the other hand (table II), disclosed a slow decrease in the rate of oxidation in relation to both age and germination for the first 4 years and then a relatively large decrease in relation to viability and senescence.

The drop in percentage oxidation is shown graphically (fig. 2). Barley, oats, and cucumber show a definite decrease in the phenolase activity in relation to age of the seeds. In wheat, on the other hand, there is an initial increase in the percentage oxidation which is subsequently followed by a decrease. It is, however, to be noted that the increase in the phenolase activity of wheat is also accompanied by an increase in the percentage germination. In the other determinations (fig. 2) the germination percentage de-

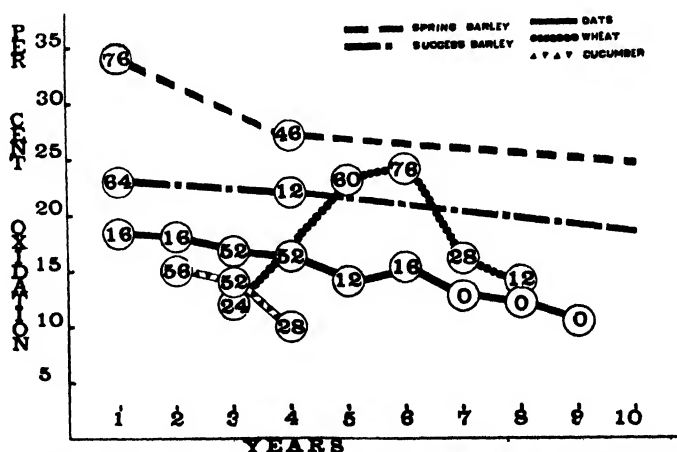


FIG. 2. Phenolase activity curves for various seeds of different ages. The numbers in the circles give the per cent. germination.

creases as the age increases in all species except oats. In this case, despite the increase in the per cent. germination, there is a decrease in degree of oxidation with age.

HARRISON (7) and other workers (8, 11) have shown that the indophenol formed in the Nadi reaction may in turn react with reducing substances

such as glutathione and hypoxanthine, being thereby reduced to its colorless leuco base. The final quantity of indophenol thus may be no reliable criterion of peroxidase activity if the concentration of reducing substances present is high. In a study of the type here reported, the utility of the Nadi reaction is not lost on this account, because reduction reactions in dormant seeds are inappreciable in comparison with those in actively growing tissues. The dilutions of the seed extracts and the brief time allowed for the reaction still further minimized reduction of indophenol.

Error of greater magnitude may possibly be introduced into the Nadi reaction by the presence of an appreciable amount of catalase, due to its decomposition of the peroxide upon which the formation of indophenol depends. Several investigations (7, 10) have shown that the inactivation of the more thermosensitive catalase at moderate temperatures actually results in an increase of indophenol by peroxidases. To test the effect of catalase on the formation of indophenol by phenolase, several samples of enzymatic seed extracts were subjected to a mild heat treatment.

Heat treatment at 52° C. for 90 minutes in a water bath without shaking (7, 10) decreased rather than increased phenolase activity in Manchuria spring barley and Arlington white spine cucumber (fig. 3). In the case of

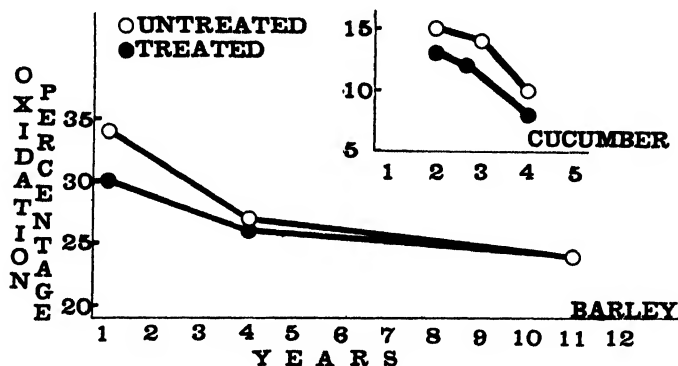


Fig. 3. Effect of heat treatment on phenolase activity.

barley of considerable age, the temperature treatment had very little effect upon the rate of oxidation. The effects of catalase in the dilutions of seed extracts employed thus appear to have been negligible.

The results of catalase determinations (table V) show a decrease in activity with age and low percentages of germination. Many experiments have been made on catalase in search of a method to determine viability, especially of seeds. SAMPIETRO (13) and CROCKER and HARRINGTON (4) show a relationship between catalase activity, age and germination. The correlation, however, is not definite enough to make reliable forecasts of the

germination percentage. The greater part of the work so far done with catalase in relation to germination apparently has been unable to evaluate precisely the effect of growth and storage conditions on enzymatic activity.

In conclusion, the Nadi reaction, involving short reaction periods and dilutions of seed extracts as above described, was found to yield concordant results in the study of phenolase activity of dry seeds of varying age. When the limitations of the Nadi reaction are circumvented, its sensitivity makes it a simple and valuable indicator of oxidase activity. Phenolase activity as herein determined showed a high degree of correlation with seed viability.

Summary

A modification of the Nadi reaction for phenolase determination is described. Phenolase activity was high in young seeds with a high percentage of germination and low in old seeds of low percentage of germination. Enzymatic material heat treated at 25° C. for 90 minutes did not show increase in phenolase activity. Phenolase and catalase activity in general were parallel in cucumber and wheat.

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USE OF EXPRESSED SAP IN PHYSIOLOGIC STUDIES OF CORN¹

J. D. SAYRE AND V. H. MORRIS

(WITH FOUR FIGURES)

In planning an extensive series of physiologic studies with corn, the need was felt for rapid, simple, yet accurate methods of making certain determinations. Plant physiologists have been using sap expressed under high pressures in various physiologic studies for many years. The apparatus and methods used and the results obtained have differed so widely that it was considered desirable to try different methods to find one that could be used satisfactorily in the investigations in mind. A description of the apparatus and procedure found satisfactory in obtaining samples of sap from vegetative parts of the corn plant suitable for some phases of the investigations, and data showing some of the limits within which such samples may be used are presented in this paper.

Apparatus and methods

A small laboratory hydraulic press (fig. 1) was used for expressing the sap from the corn tissues. A cage to contain the tissues, similar to that described by MEYER (2), was constructed so that the 25,000 pounds total working load exerted a pressure of 5,000 pounds per square inch on the tissue in the cage. The apparatus was arranged so that the sap obtained was filtered. The filter consisted of a layer of linen cloth and a layer of 100-mesh copper sieve wire placed over a perforated steel disk in the bottom of the cage. The disk was perforated with one-sixteenth inch holes, arranged radially in rows, with grooves on the lower side of the disk for drainage. Filtered through this, the sap contained no cell residue and was practically free from suspended material. The cage was sealed liquid tight by a rubber stopper placed above the tissue.

A uniform time of draining was found necessary for obtaining comparable results. If many samples are to be examined this time must be a minimum. In these experiments five minutes were allowed for draining, during which time the pressure was maintained at 25,000 pounds; this was long enough to express practically all of the obtainable sap.

In some of the experiments it was desired to test successive portions of the sap. The successive 10-cc. samples of sap from 100 grams of tissue were collected in separate 10-cc. graduated cylinders. The same process

¹ Based on investigations cooperative between the Ohio State Agricultural Experiment Station and the Office of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

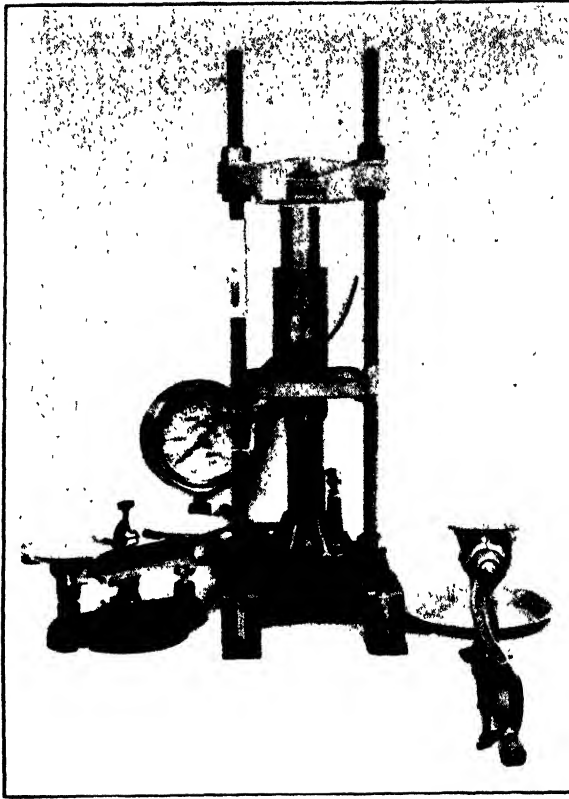


FIG. 1. Equipment used for obtaining samples of expressed sap from corn tissue.

was repeated with two other 100-gram samples of tissue and the corresponding portions from the three expressions were mixed to provide samples for examination.

Total solids in the sap were determined with the refractometer, GORTNER and HOFFMAN (1). The total reducing power of the sap was determined by a modification of the QUISUMBING-THOMAS method (3) after inversion of the sucrose by invertase. In analyzing the plant tissue for sugars for comparison with estimates based on the sap analyses the samples, which had been preserved in alcohol, were extracted with 80 per cent. alcohol and the total reducing power determined as above. In the comparisons shown this is designated as the "standard" method in contrast to the "sap" method.

Results

The experiments fall naturally into studies of (1) factors involving the amount of sap or water expressed, (2) the sugars and total solids in the sap

and in successive portions thereof, and (3) the relation between the sugars in the sap and in the tissue. The results with sucrose and with free reducing sugars were so alike that they have been combined, and only total sugars are reported.

THE AMOUNT OF SAP EXPRESSED

Among the factors affecting the amount of sap which could be expressed studies were made of the preliminary treatment of the tissue, the size of sample, and the kind of tissue and its moisture content.

Effects of preliminary treatment.—Various methods of treating the tissue prior to expressing the sap have been used by other workers. These methods have been summarized by MEYER (2). Here, preparation by grinding the tissue with a food grinder (fig. 1) and mincing it, with and without freezing it to -9° C. in each case were compared. The grams of sap recovered from 100 grams of blade tissue of Burr-Leaming corn and of orchard grass after the different treatments are shown in table I.

TABLE I

THE AMOUNT OF SAP EXPRESSED FROM BLADE TISSUE OF CORN AND OF ORCHARD GRASS
FOLLOWING DIFFERENT TREATMENTS
(AVERAGE OF THREE DETERMINATIONS)

BLADE TISSUE FROM	AMOUNT OF SAP FROM 100 GRAMS OF TISSUE			
	GROUND	GROUND AND FROZEN	MINCED	MINCED AND FROZEN
Corn	gm. 71	gm. 72	gm. 26	gm. 45
Orchard grass	53	58	13	33

More sap was recovered after grinding only than after mincing, either with or without freezing. Moreover, the additional amount of sap recovered after grinding and freezing the tissue, above that obtained after grinding only, was negligible. Grinding is more simple and rapid, and omission of freezing avoids any danger of change in the colloids that might result from that process. Finally, as will be shown later, successive portions of sap were more nearly alike after grinding than after mincing. Grinding seemed to be the preferable method of preparation and accordingly was used in the succeeding experiments reported in this paper.

THE INFLUENCE OF SIZE OF SAMPLE.—Using uniform pressures and times of draining, samples of 50, 100, and 150 grams were compared. Representative data for different tissues are given in table II.

TABLE II

PERCENTAGE OF TOTAL WEIGHT EXPRESSED AS SAP FROM DIFFERENT SIZED SAMPLES OF DIFFERENT CORN TISSUES

KIND OF TISSUE	PERCENTAGE TOTAL WEIGHT EXPRESSED FROM SAMPLES OF:		
	50 GRAMS	100 GRAMS	150 GRAMS
	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>
Stem	82	80	80
Blade	78	72	66

Larger percentages of sap were removed from the smaller samples, particularly of the blade tissue. As will be shown later, the sap remaining in the press cake is similar in composition, in certain respects, to that expressed. Accordingly, the 100-gram sample was used in our experiments in order to provide adequate quantities of sap and moderately high expression. For some purposes it probably would be better to obtain the necessary sap from two smaller samples.

KIND OF TISSUE AND MOISTURE CONTENT.—The amount of sap removed by pressure from blade and stem tissue of Burr-Leaming corn at different stages in its development is shown graphically in figure 2. The amount of sap obtained has been expressed as cubic centimeters of water (by subtracting the total solids from the weight of sap obtained) for comparison with the absolute moisture content of the tissue.

The amount of water which was expressed naturally was highly correlated with the amount in the tissue. This correlation was not perfect, however, a larger amount of water remaining in the blade tissue (20 to 27 cc.) than in the stem tissue (8 to 10 cc.). In the blade tissue, moreover, the absolute amount left after pressure extraction was larger in the samples obtained later in the season and in which the original moisture contents were less.

SOLIDS AND SUGARS IN SUCCESSIVE PORTIONS OF SAP

If successive portions of sap expressed from a given sample of tissue have the same composition with respect to certain constituents, the sap remaining in the press cake may be assumed also to have this composition. The total solids, sucrose, and free reducing sugars were determined in suc-

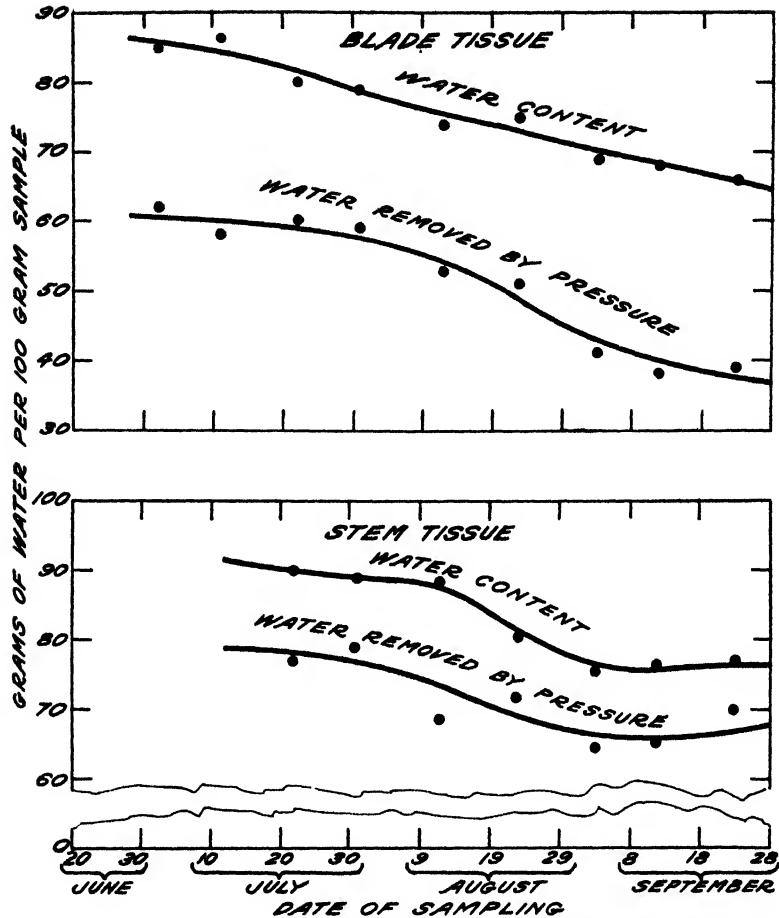


FIG. 2. The water content and the amount of water removed by pressure, from blade and from stem tissue of Burr-Leaming corn, summer 1929.

cessive portions of sap from different tissues after grinding only and after mincing. The data from a representative experiment are shown in table III.

The solids content decreased in the successive portions of sap from all samples. The decrease was greater with blade than with stem tissue, and greater with minced than with ground tissue. The maximum decrease was from 6.2 per cent. of solids in the first portion to 4.2 per cent. in the last portion of sap from minced blade tissue. The corresponding decrease for the ground tissue was from 11.1 to 9.1 per cent. The differences with the stem tissue were similar though usually smaller.

The percentages of sugars were practically the same in the successive portions from ground tissue, whether stem or blade. With the minced

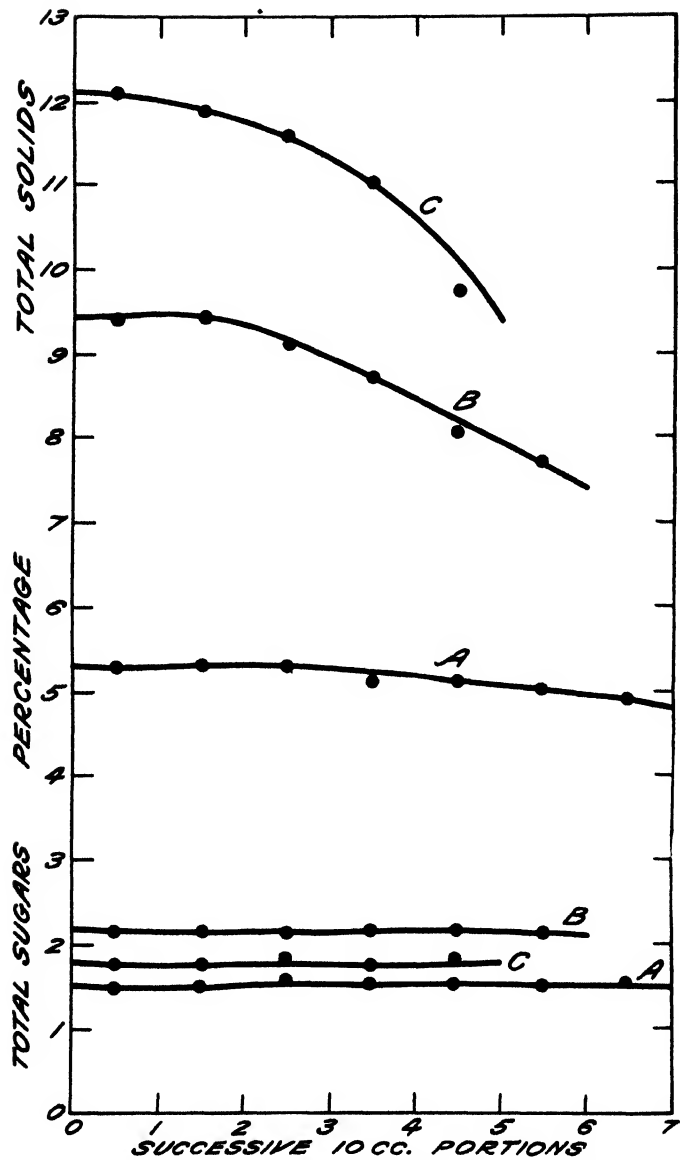


FIG. 3. Percentage of total solids and total sugars in successive 10-cc. portions of sap from blade tissue of Burr-Leaming corn. A = 86 per cent. moisture, B = 74 per cent. moisture, C = 70 per cent. moisture.

TABLE III

PERCENTAGES OF TOTAL SOLIDS AND TOTAL SUGARS, IN SUCCESSIVE 10-CC. PORTIONS OF EXPRESSED SAP OF DIFFERENT CORN TISSUES AFTER DIFFERENT TREATMENTS

PORTION	GROUND				MINCED			
	STEM		BLADE		STEM		BLADE	
	SOLIDS	SUGARS	SOLIDS	SUGARS	SOLIDS	SUGARS	SOLIDS	SUGARS
	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>
1	12.9	12.1	11.1	2.9	9.9	10.1	6.2	2.4
2	12.9	12.2	11.3	2.9	9.9	10.0	4.2	1.1
3	12.8	12.1	10.5	2.8	9.3	9.1
4	12.8	12.1	10.3	2.9	8.7	8.0
5	12.8	12.3	9.6	2.7	7.9	7.5
6	12.8	12.4	9.1	2.9		
7	12.2	12.2				
Last drop	11.8		8.5		7.5		4.0	...
Duplicate 100-gm. sample	12.9	12.1	10.4	2.9	9.4	9.3	5.2	1.8

tissues, the sugars decreased in the successive portions similarly to the solids. Grinding the tissue resulted not only in obtaining larger percentages of solids in the sap but also in obtaining sap having the same sugar content in successive portions.

The total solids and sugars in successive portions of sap from stem and from blade tissues of corn after grinding were determined at three different stages of growth during the season. The percentages in the blade tissues, containing 86, 74, and 70 per cent. of moisture respectively, are shown graphically in figure 3.

The total solids decreased with the successive portions, the decrease being the largest for the drier (more advanced) tissue and least for the tissue containing the most moisture. Similar differences, but much smaller ones, were found in the successive portions of sap from stem tissue. The total sugar content was the same in the successive portions of sap for both kinds of tissue at all stages of development.

RELATION BETWEEN THE SUGAR CONTENT OF SAP AND TISSUE

The sugar content of successive portions of sap expressed from ground tissue was the same for both kinds of tissue and for three stages of development. It seemed probable, therefore, that the sugar content of the tissue could be calculated with more or less accuracy from the sugar content of the expressed sap and the moisture content of the tissue.

During a seasonal study of the distribution of sugars in the corn plant, 58 pairs of samples for sugar determinations were taken. The sugar con-

tent of one sample of each pair was determined by the "standard" alcoholic extraction method and the sugar content of the other was estimated from the sap analysis and the moisture content. In calculating the sugar content of the tissue from sap analysis, no correction was made for the specific gravity and total solids content of the sap. Subsequent data have shown that without this correction the tendency is for the sugar content of the tissue estimated from sap analysis to be slightly low. The samples comprised blade, sheath, and stem tissues from Burr-Leaming and Medina Pride corn, during the period from July 2 to September 24.

The average sugar content of the three tissues from each kind of corn as determined by the two methods is shown in table IV, and the detailed comparison for Burr-Leaming tissues is shown graphically in fig. 4.

TABLE IV

PERCENTAGE SUGAR CONTENT OF CORN TISSUE (1) DETERMINED BY THE STANDARD METHOD, AND (2) CALCULATED FROM THE SUGARS IN EXPRESSED SAP AND THE MOISTURE IN THE TISSUE

TISSUE	BURR-LEAMING				MEDINA PRIDE			
	NUMBER OF SAMPLES	METHOD		DIFFER- ENCE	NUMBER OF SAMPLES	METHOD		DIFFER- ENCE
		STANDARD	SAP			STANDARD	SAP	
		<i>per cent.</i>	<i>per cent.</i>					
Blade	9	2.14	2.06	- 0.08	9	2.11	2.26	+ 0.15
Sheath	17	4.79	4.50	- 0.29	7	4.56	4.40	- 0.16
Stem	14	7.82	7.71	- 0.11	12	7.98	7.42	- 0.56
All	30	5.41	5.27	- 0.14	28	5.24	5.00	- 0.24
All, both varieties	58	5.33	5.14	- 0.19				

The maximum difference between methods was a deficiency of 0.9 per cent. in stem tissue of Medina Pride having a sugar content of 9.1 per cent. (standard method), or a difference of 10 per cent. On the other hand, a sample of blade tissue of the same variety, containing 2.5 per cent. of sugar by the standard method, was estimated as containing 3.2 per cent. by the sap method. The difference here is smaller absolutely but larger relatively, being 28 per cent. of the total. In general, the sugar content indicated by the sap method tended to be lower, the mean difference for the 58 pairs of samples being -0.19 ± 0.034 per cent.

As an additional criterion, duplicate samples of tissue corresponding to 12 of these 58 were analyzed by the standard method and the values compared with the original determinations. These samples comprised different tissues at different stages of growth with a range in sugar content of from 2.68 to 10.50 per cent. The average difference between the paired samples

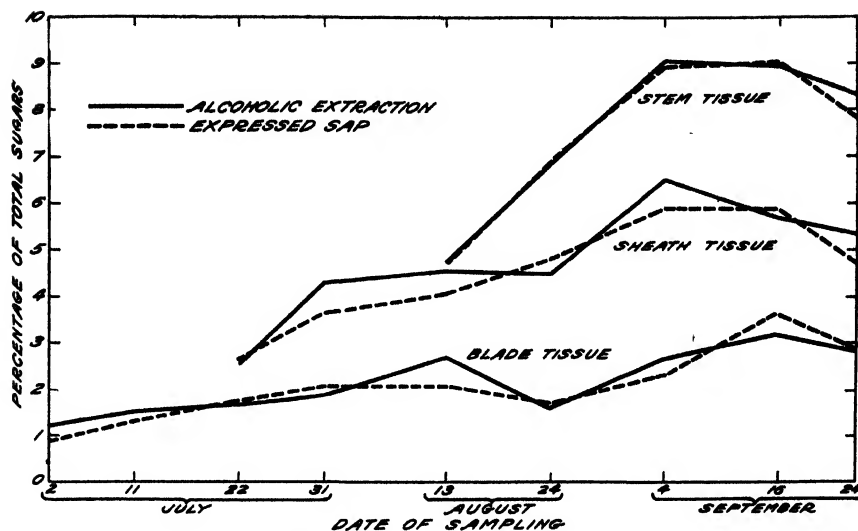


FIG. 4. Percentage of total sugars in blade, sheath, and stem tissue of Burr-Leaming corn obtained by the alcoholic extraction of the tissue and by the expressed sap method.

was 0.26 ± 0.072 . The results with the two methods accordingly were no more inconsistent for 58 samples than were those by the standard method for 12 samples, and the difference of -0.19 ± 0.034 , though significant statistically, must be considered unimportant in a comparison of the two methods.

The agreement between methods over the entire range of the 58 samples is measured best by the coefficient of correlation between the corresponding values. This coefficient is 0.9913, indicating excellent agreement, with only 1.73 per cent. of the variation (as variance) independent. The data indicate, then, that the sugar content of corn tissues may be estimated from the analysis of sap expressed as described, with little or no error in excess of that to be expected from duplicate samples analyzed by the standard method.

Summary

1. The amount of sap expressed from corn tissue under a uniform pressure and with a uniform time for draining the cage was influenced by the preliminary treatment of the tissue, by the size of sample, and by the kind of tissue and its moisture content as affected by its stage of development.

2. The total solids content differed in successive portions of sap expressed from corn tissue, the size of the differences varying with the treatment preliminary to expression, the kind of tissue and its moisture content.

3. The sucrose and free reducing sugars, reported together here as total sugars, were constant for successive portions of sap expressed after grinding the tissue, but decreased in successive portions from minced tissue.

4. The percentages of total sugars in three kinds of fresh corn tissue from two varieties, at different stages of growth, as determined by the standard method and from the sap expressed after grinding, were in excellent agreement, the coefficient of correlation being 0.9913. The values by the two methods were in as good agreement as those for 12 pairs of samples for which sugars were determined by the standard method.

5. Expressing the sap from a 100-gram sample of ground fresh corn tissue under a pressure of 5,000 pounds per square inch, at the same time forcing the sap through a filter, and allowing a uniform time (5 minutes) for draining, provided samples of sap that were satisfactory for certain physical and chemical examinations.

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EFFECT OF PETROLEUM OILS ON THE RESPIRATION OF BEAN LEAVES

JESSE GREEN AND ARNOLD H. JOHNSON

(WITH FIVE FIGURES)

The injurious effect of petroleum oils on fruit trees is a serious objection to their use as insecticides. The damage appears in a great variety of ways (10). Trees sprayed in dormant stage show injury during the following spring as a wrinkling or withering of the bark and retardation of leaf formation. The damage to sprayed leaves generally appears as oil soaked spots or a more acute drying or burning of the leaf.

The exact physiological actions accompanying injury from oil are not well known. The problem appears to be divided into two major possibilities; plugging of stomata and poisoning of cells. In either case respiration should be affected. It has been the purpose of this work to determine the change in rate of respiration of plants which have been sprayed with oil.

Mechanical injury has been shown by RICHARDS (9) and others (3) to increase the rate of respiration. Toxic and anesthetic materials at certain concentrations are also expected to increase the respiration of plants (2, 8).

KNIGHT, CHAMBERLIN and SAMUELS (6) have determined the effect of a viscous, highly refined oil in the rate of respiration of citrus trees. Their results show that respiration is greatly increased and remains abnormally high for a long period of time after treatment with this type of oil.

Believing that respiration is generally increased when a plant is injured, an attempt has been made in this study to show the injurious effect on the respiration of bean leaves by the different types of oil used as sprays.

Methods

PLANTS

Dandelion and potato leaves were first used, but later bean leaves were found to give more uniform results. Three- to six-gram samples of bean leaves were taken and the stems placed in water as soon as they were cut. Four samples were used for checks and four were sprayed with oil.

SPRAYING

The spraying was done with an atomizer operated with compressed air (figure 1). It was calibrated before each determination to deliver a definite

amount of oil. This was done by spraying weighed filter papers and then weighing after spraying to determine the amount of oil delivered. The leaves were then sprayed with the same nozzle adjustment, temperature and air pressure. The time of spraying the leaves was adjusted to apply 10 milligrams of oil on a circular area 7 centimeters in diameter, which was the size of the filter-paper used for calibration.

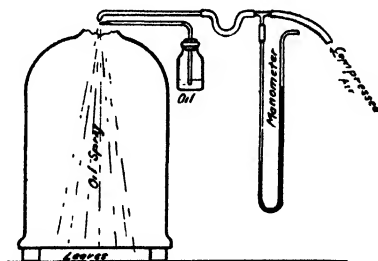


FIG. 1. Apparatus for spraying leaves.

Considerable preliminary work was done on the effect of the amount of oil within the range between very light spraying and dipping the leaves in oils. The amount finally selected gave a visible coating of oil on the leaves and, when calculated, is 0.26 milligram of oil per square centimeter.

OILS

Petroleum oils that are used for spraying are usually of the lubricating type. Nine kinds of oils were selected for experimental use. They had widely varying characteristics, as is shown in table I. The oils were ap-

TABLE I
OILS USED IN RESPIRATION EXPERIMENTS

No.	MANUFACTURER	MANUFACTURER'S NO.	TRADE NAME	SULPHONAT- ABLE RESIDUE	VISCOSITY
				<i>per cent.</i>	<i>deg.</i>
1	Standard	13604R	100 Pale	15	100-110
2	Standard	13605R	Calol red engine	44	220
3	Standard	13606R	Oronite technical	5	100
4	Standard	13607R	Mineral seal	10	50
5	Standard	14510R		35	110
10	Standard	13240R		39.7	140
13	Shell	R-L-99		18.3	72
21	Shell	106		14.4*	53
24	Sonneborn & Sons		Amelie	none	67

* SO₂ treated.

plied in the pure state. Emulsions were not used because they would have introduced too many variable factors for this problem.

METHOD OF MEASURING RESPIRATION

A simple method for measuring respiration was devised, following the plan used by LUND (7). After the leaves had been sprayed they were fastened, by means of a rubber band, to a stopper on the tube *a*, figure 2, and were placed in a one-half gallon Mason jar made of white glass. The tube *a* extended downwards about one-half the depth of the jar, and had another stopper on the lower end to keep the leaves apart.

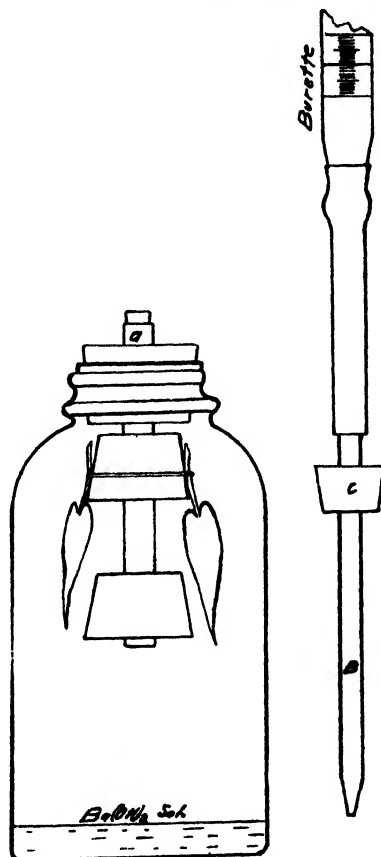


FIG. 2. Respiration jar and burette for measuring and titrating $\text{Ba}(\text{OH})_2$ solution.

A titration set was prepared with long tips *B* that would reach down through the tube *a*. One burette was used for adding the $\text{Ba}(\text{OH})_2$ solution which absorbed the CO_2 from the plants, and another for titrating the excess $\text{Ba}(\text{OH})_2$ with HCl at regular intervals. The tips were selected so as

to fit the tube *a* closely, which was kept stoppered except during titrations. The tip had a rubber stopper *c* near the top which was held down tightly against the tube *a* while titrations were being made to exclude as much outside air as possible.

When the leaves were in place, 25 to 50 cc. of approximately 0.010 N $\text{Ba}(\text{OH})_2$ were measured into the jar. Two blank jars without leaves were prepared in exactly the same way, and all were placed on a shaker in an asbestos-lined, constant temperature box maintained at 28°C ., figure 3. The shaker was operated with a small motor with a fan on its shaft for circulating the air in the constant temperature box. A very slight shaking motion was sufficient to rock the solution and to circulate the gases in the respiration jars, thus continually exposing a new surface of $\text{Ba}(\text{OH})_2$ to the CO_2 .

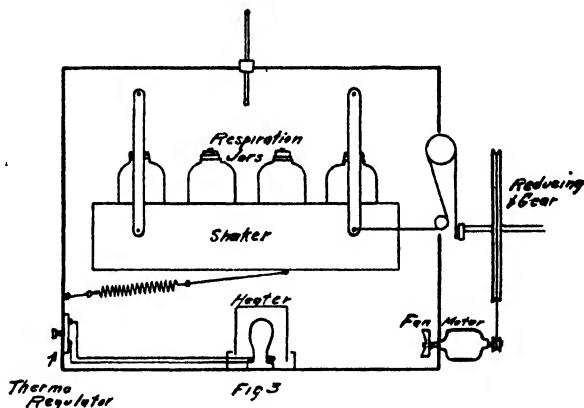


FIG. 3. Respiration jars on shaker in constant temperature box.

Titration were made at two-hour intervals with exactly 0.005 N HCl. Phenolphthalein was used as the indicator. In the preliminary work the indicator was added in alcoholic solution in the ordinary way, but, to avoid the effect of the alcoholic vapors on the leaves, the phenolphthalein was later placed directly in the standard $\text{Ba}(\text{OH})_2$ solution. When the $\text{Ba}(\text{OH})_2$ solution was prepared a standard aqueous solution of the phenolphthalein was added until the desired color was obtained. The effect of a small amount of alcoholic vapor in the closed system of the respiration jars was not known, but the probabilities are that it is considerable.

In calculating the results the titrations of the blank jars were subtracted from those of the samples, the differences representing the CO_2 given off from the leaves. One cc. of 0.005 N HCl equals very nearly 0.11 mg. of CO_2 .

Discussion

In the closed system for respiration one of the first questions that arises is the effect of the change in concentration of gases; however, the change is very small. The oxygen in a one-half gallon jar can be decreased only very slightly by 3 to 6 grams of leaves in 10 hours. According to KOSTRYCHEV (5) the concentration of oxygen has very little effect on the respiratory process as long as some oxygen is present. It is stated that a reduction to one-half the normal concentration of oxygen does not change the rate of respiration.

The concentration of CO_2 is kept constant in the closed system, as the moving $\text{Ba}(\text{OH})_2$ absorbs it as fast as it is formed. The concentration of water vapor may be important. KELLEY (4) states that a high humidity favors oil damage, while on the other hand YOUNG (10) finds that in field work the greatest damage from oil occurs when trees are suffering from drought. In the method used, the leaves were in a saturated atmosphere and seemed to go through the ten-hour tests without any apparent injury.

The effects of light and temperature were reduced to a minimum. The constant temperature box was heated with an electric light bulb, automatically controlled. This was covered to eliminate the effect of light in the box. The leaves were in total darkness except for the 3 to 5 minutes required to make titrations at the two-hour periods. Any effect of light and temperature changes was very nearly the same on all the samples.

The age and vitality of the leaves were found to have a great influence on their respiration. Young leaves respire at a more rapid rate than older ones, per gram of leaf. For this reason much care was used in selecting the samples. The curves show that in some determinations the average rate of respiration was much higher than in others even though samples were selected from plants of very nearly the same age.

Because of the variability of samples, several leaves were used in each test and four checks were always run against four treated samples. At the conclusion of the work it was found that even this number was not sufficient. A calculation of the variability of two check samples against two other checks of the same series, showed that the average variability for a group of 17 determinations was 7.5 per cent. It is apparent that the variability of four checks compared with four others, as in the case of the experiments, would be less, probably of the order of one-half to one-third less, making the actual error of the work 4 to 6 per cent.

The variation of the treated samples was much greater than the normals. The average variation of the treated samples, when computed in the same way, was 11.5 per cent., which indicates that respiration was greatly affected by oil.

In the beginning, increasing amounts of oil were expected to gradually accelerate respiration until a maximum should be reached, after which more oil would reduce the rate of respiration.

A series of experiments, using kerosene, was performed to show this effect, because kerosene was expected to be more injurious than lubricating oils. Four experiments were conducted in which the amount of kerosene was doubled each time and finally one set of samples was dipped in kerosene. The results are shown in table II and in figure 5, curves nos. 18, 19, 20, 21 and 22. The respiration rate increased quite regularly with increasing amounts of oil until a maximum was reached after which more oil caused decreased respiration. When the leaves were dipped in oil their respiration dropped to 52.2 per cent. of the normal rate.

TABLE II

RESPIRATION OF NORMAL BEAN LEAVES AND BEAN LEAVES SPRAYED OR DIPPED IN KEROSENE

CURVE NO.	TREATMENT	CO ₂ GIVEN OFF, MEASURED AT 2-HOUR INTERVALS					TOTAL CO ₂ FOR 8 HOURS	DIFFERENCE	GAIN	LOSS
		mg.	mg.	mg.	mg.	mg.				
18	Sprayed 1 min.	1.34	1.36	1.57	1.43	1.40	7.10			
18	Normal	0.69	1.13	1.24	1.05	1.02	5.13	1.97	38.4	
19	Sprayed 2 min.	1.23	1.37	1.58	1.47	1.51	7.16			
19	Normal	1.01	1.11	1.22	1.08	1.04	5.46	1.70	31.1	
20	Sprayed 4 min.	1.28	1.66	1.85	1.60	1.54	7.93			
20	Normal	1.14	1.33	1.28	1.04	0.91	5.70	2.23	39.1	
21	Sprayed 8 min.	1.34	1.65	1.63	1.29	1.34	7.25			
21	Normal	1.14	1.45	1.17	1.05	1.04	5.85	1.40	23.9	
22	Dipped in kerosene	1.15	0.62	0.38	0.47	0.46	3.08			
22	Normal	1.73	1.59	1.25	1.32	1.28	7.17	4.09		57.0

The lubricating oils used may be divided into two classes: light colored, and dark colored. The dark colored oils were usually more viscous and contained greater amounts of sulphonatable residue than light colored oils.

The term sulphonatable residue designates that portion of an oil that can be extracted with strong sulphuric acid under strictly analytical specifications. It consists largely of unsaturated hydrocarbons, sulphur, nitrogen and aromatic compounds. There is evidence that some of these sulphonatable compounds are toxic to plants. GRAY and DE ONG (1) have shown from field tests that injury increases very closely with the percentage

of sulphonatable residue. Perhaps the unsaturated hydrocarbons are responsible for the trouble, but many of the compounds in the sulphonatable residue are unstable and may have deleterious effects on living plants.

A comparison of the effect of oils of low sulphonatable residue, figures 4, 5, oils nos. 3, 4 and 24, with those of high sulphonatable residue,

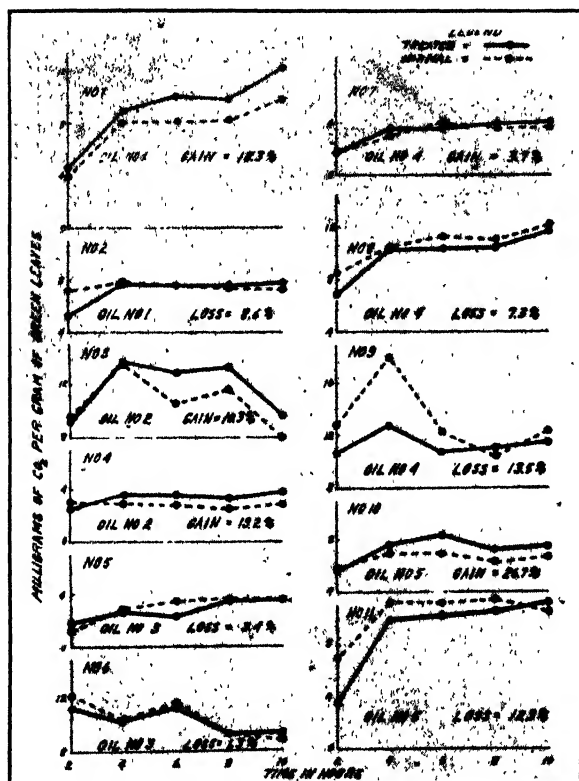


FIG. 4. Curves showing the effect of oil on the respiration of bean leaves.

nos. 2, 5 and 13, show that the former group caused an average loss of 5.8 per cent. in rate of respiration in 7 determinations, while the latter caused an average gain of 8.1 per cent. in 6 determinations. By arranging all of the light colored oils of less than 16 per cent. sulphonatable residue in one group and the darker colored oils of more than 16 per cent. sulphonatable residue in another group, a similar difference is shown. In 8 determinations the light colored oils caused an average loss of 5.0 per cent. in rate of respiration, while, in 9 determinations, the dark oils caused an average gain of 7.5 per cent. The analytical results are shown in table III and are graphically represented in figures 4 and 5, curves 1 to 17.

TABLE III
RESPIRATION OF OIL SPRAYED AND NORMAL BEAN LEAVES

CURVE NO.	OIL NO.	CO ₂ GIVEN OFF, MEASURED AT 2-HOUR INTERVALS					TOTAL CO ₂ FOR 8 HOURS	DIFFERENCE	GAIN	Loss
		mg.	mg.	mg.	mg.	mg.	mg.	mg.	per cent.	per cent.
1	1 Sprayed	0.46	0.90	1.01	0.99	1.23	4.59			
1	Normal	0.39	0.81	0.81	0.82	0.99	3.82	0.77	18.3	
2	1 Sprayed	0.13	0.37	0.36	0.36	0.38	1.60			
2	Normal	0.33	0.39	0.36	0.34	0.33	1.75	-0.15		8.6
3	2 Sprayed	0.90	1.38	1.29	1.33	0.96	5.86			
3	Normal	0.95	1.35	1.05	1.16	0.80	5.31	0.55	10.3	
4	2 Sprayed	0.25	0.36	0.36	0.33	0.38	1.68			
4	Normal	0.30	0.29	0.28	0.25	0.29	1.41	0.27	19.2	
5	3 Sprayed	0.20	0.27	0.23	0.35	0.36	1.41			
5	Normal	0.10	0.28	0.35	0.37	0.36	1.46	-0.05		3.4
6	3 Sprayed	1.15	1.02	1.13	0.92	0.94	5.16			
6	Normal	1.22	1.04	1.17	0.92	0.88	5.23	-0.07		1.3
7	4 Sprayed	0.16	0.36	0.36	0.40	0.42	1.70			
7	Normal	0.16	0.30	0.42	0.37	0.39	1.64	0.06	3.7	
8	4 Sprayed	0.68	1.03	1.04	1.05	1.18	4.98			
8	Normal	0.84	1.05	1.13	1.12	1.23	5.37	-0.39		7.3
9	4 Sprayed	1.07	1.28	1.07	1.11	1.15	5.68			
9	Normal	1.23	1.81	1.23	1.05	1.25	6.57	-0.89		13.5
10	5 Sprayed	0.16	0.37	0.44	0.33	0.36	1.66			
10	Normal	0.19	0.30	0.30	0.24	0.28	1.31	0.35	26.7	
11	5 Sprayed	0.31	0.99	1.02	1.06	1.12	4.50			
11	Normal	0.68	1.12	1.12	1.15	1.06	5.13	-0.63		12.3
12	5 Sprayed	0.70	0.86	1.02	0.89	0.88	4.35			
12	Normal	0.70	0.90	1.00	0.90	0.89	4.39	-0.04		0.9
13	10 Sprayed	0.48	0.72	0.78	0.69	0.66	3.33			
13	Normal	0.46	0.67	0.69	0.61	0.62	3.05	0.28	9.2	
14	13 Sprayed	0.29	0.90	1.08	0.97	0.97	4.21			
14	Normal	0.30	0.91	1.05	0.90	0.83	3.99	0.22	5.5	
15	21 Sprayed	0.72	1.05	1.00	0.93	0.79	4.49			
15	Normal	0.69	1.02	0.99	0.94	0.83	4.47	0.02	0.4	
16	24 Sprayed	0.72	0.96	0.82	0.86	0.71	4.07			
16	Normal	0.71	1.04	0.88	1.02	0.78	4.43	-0.36		8.1
17	24 Sprayed	0.56	0.81	0.91	0.73	0.84	3.85			
17	Normal	0.64	0.96	1.04	0.80	0.88	4.32	-0.47		10.9
		Total							93.3	66.3

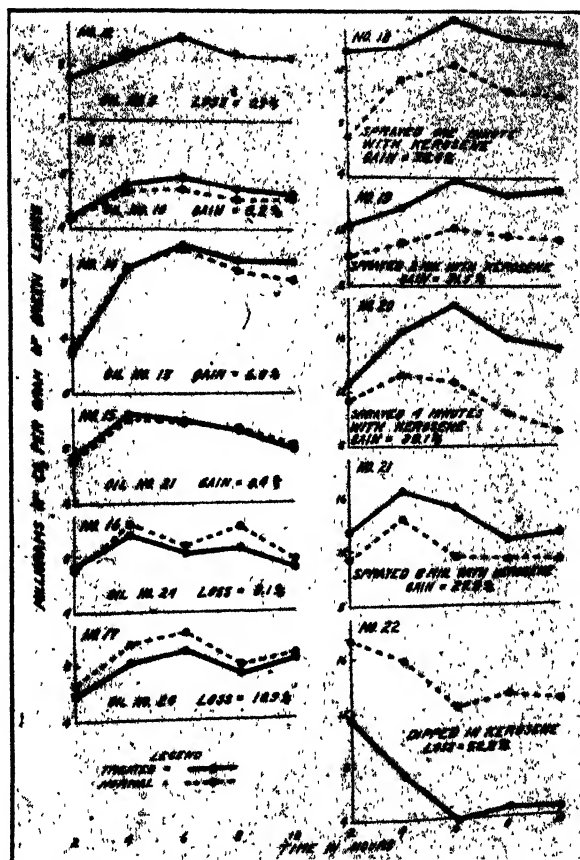


FIG. 5. Left, effect of lubricating oil on the respiration of bean leaves. Right, effect of varying amounts of kerosene on the respiration of bean leaves.

This evidence does not prove that the difference was due only to sulphonatable residue. There is some evidence that sulphonatable residues alone increase respiration. Oil no. 3 is a white oil with a sulphonatable residue of 5 per cent. and a viscosity of 100. Oil no. 5 is a yellow oil with 35 per cent. sulphonatable residue and a viscosity of 110. The difference of viscosity is small and the difference in sulphonatable residue is comparatively large. For 2 determinations oil no. 3 caused an average loss of 2.4 per cent. in CO₂ respired and for 3 determinations oil no. 5 caused an average gain of 4.3 per cent.

The indications are that the sulphonatable residue of an oil causes an increase in the rate of respiration of bean leaves and that the more highly refined oils cause a decrease in the rate. Satisfactory explanations for

these two actions are difficult. The reactions leading up to the taking in or giving off of CO_2 from plants are very involved but in their simplest expression may be considered mainly as oxidation and reduction reactions.

An increase in the rate of CO_2 production over the normal is spoken of in this work as increased respiration. As it has been shown that the oils with high sulphonatable residues cause an increase in respiration it follows that the sulphonatable compounds must in some way favor oxidation in the plant. The light colored oils with low sulphonatable residue cause a decrease in respiration which indicates that these oils have favored carbon reduction or the greater use of CO_2 in the plant itself.

The exact means by which oxidation and reduction are promoted is as yet too difficult for explanation. These two actions are, no doubt, affected in a physical way by the oils whenever there is any interference with the exchange of gases through the stomata of the leaves. A change in the rate of flow of gases through their regular channels would cause changes in the concentration of the reacting material in the leaves at the points of action and may thus have a decided influence on the products formed. However, the chemical action of the compounds in the oil on the plant juices and cells is perhaps the more fundamental cause for changes in the rates of oxidation or reduction followed by changing rates of CO_2 production.

In any event the two tendencies, oxidation and reduction, are well balanced against each other in leaves. This balance is sustained by many factors. It was found that at least some of the factors were not even known and could not be controlled. With all the precautions taken to regulate temperature, light, etc., it will be seen that on some occasions either type of oil caused an increase or a decrease in the respiration rate.

In the case of oil no. 4 (a light oil of low sulphonatable residue) 3 determinations were made and 1 of these showed an increase, which is contrary to the rule. Also, with oil no. 5 (a dark colored oil of high sulphonatable residue) 3 determinations were made and 2 of these showed decreased respiration, which is decidedly different from the general trend of all the oils of this type. It is obvious that there are variables that are not yet under control and it is only by making a large number of determinations that any significance can be attributed to the results.

Both types of oil when used in high concentrations cause damage in the field (10). Oil no. 24 is highly refined and has no sulphonatable residue but when used in high concentrations it has caused injury in orchard tests. It may, however, be said that the oils with small amounts of sulphonatable residue cause less injury to foliage than those containing large amounts (10). Although this work was begun with the general impression that increased respiration was a sign of injury the question now arises regarding

the relation of decreased respiration to injury. A positive answer can not now be given but the hypothesis will be advanced that any change in rates of respiration from the normal may be looked upon as the result of injury.

Summary

When bean leaves were sprayed with the dark petroleum oils containing more than 16 per cent. of sulphonatable residues, their rates of respiration were increased. In 9 determinations using five dark colored oils the average increase in CO_2 respired by the sprayed leaves over the normal was 7.5 per cent.

Light colored oils of less than 16 per cent. sulphonatable residue caused a decrease in the rate of respiration of bean leaves. In 8 determinations using four different light colored oils an average loss of 5.0 per cent. in CO_2 respired occurred.

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STRANGULATION OF COTTON ROOTS¹

J. J. TAUBENHAUS, W. N. EZEKIEL AND H. E. REA

(WITH TWO FIGURES)

In 1923, specimens of a peculiar and, until recently, puzzling cotton root trouble were received. The specimens were of cotton plants with well-developed tops but with extremely shallow and very greatly reduced root systems (fig. 1 *a*). There was no indication of attack by fungi or bacteria, nor of injury by insects, rodents, or farm implements, to account for the shortened roots; yet it was difficult to explain how such large plants could have developed without more extensive root systems. These specimens were from widely separated points in Texas: Mercedes, Eloise, El Campo, and Chillicothe. We have had no further record of this condition on cotton in this state until the past season. However, affected cotton plants were received in 1924 from W. E. AYRES, then Assistant Director of the Delta Branch Experiment Station at Stoneville, Mississippi; from Dr. V. H. YOUNG, of the Arkansas Agricultural Experiment Station; and from Burdette, Arkansas.

During the summer of 1929, many specimens were again received from cotton planters in different parts of the State, including the four places where affected plants had been found in 1923. Affected plants were also collected by the writers from cotton fields near Itasca, Buna, Cameron, Palmer, and Von Ormy. It was possible for the first time to study the root systems of affected plants in place in the field. Cotton growers referred to such cotton plants as "rootless cotton," "flat-foot," "club-foot," and "bumble-foot." These terms are undesirable since the affected plants actually possessed well-developed roots, which, however, were constricted just below ground. The name "root strangulation" appears more appropriate and is suggested. Cotton root strangulation has appeared only sporadically, and is of economic importance only in limited areas. In affected fields, in 1929, losses from dead plants ranged from 3 to 8 per cent. of the stand. Many of the affected plants died early in the season, before the bolls were fully developed, while there were often one to five normal bolls on other plants.

Description

The specimens received during 1923 and 1924, and affected plants studied in the field in 1929, were in general similar in appearance. Some

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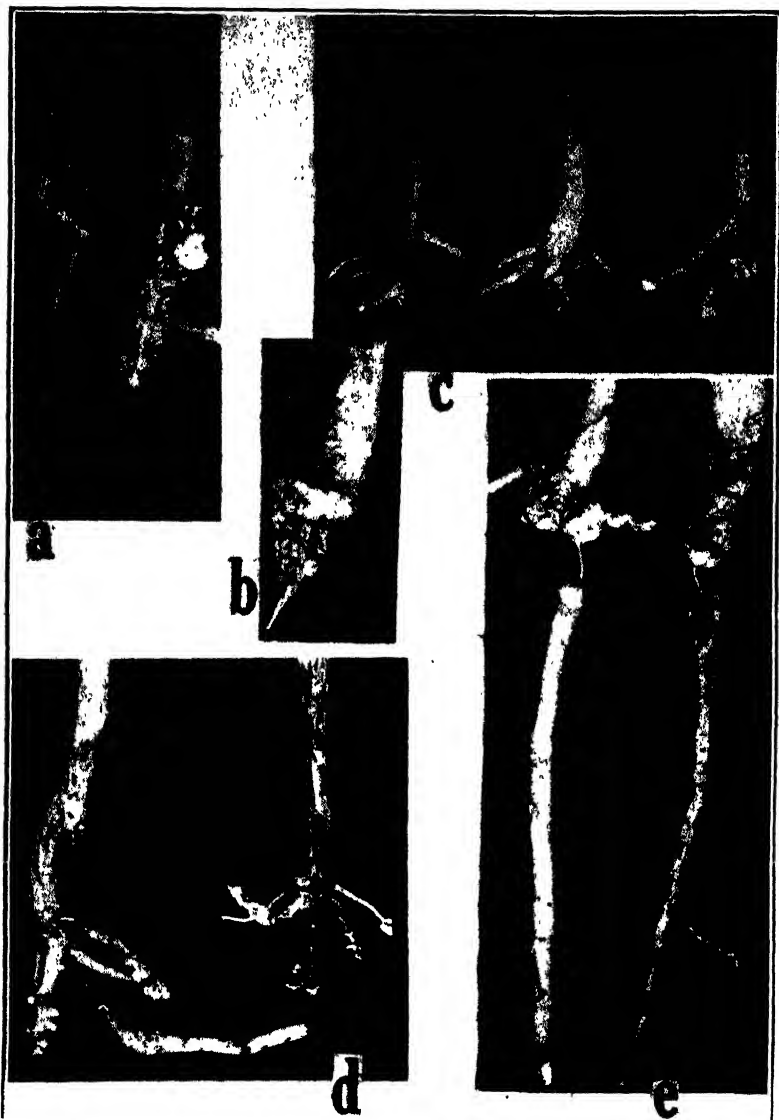


FIG. 1. Root strangulation of cotton, *a* and *b*, upper parts of tap-roots as pulled from the ground, showing roots enlarged just below surface of ground and tapering to needle-like tips; *c*, tap-roots and lateral roots showing strangulation; *d*, strangulated roots with bead-like calluses; and *e*, root systems of strangulated cotton plants as demonstrated by excavations, showing the strangulated areas connecting the upper and lower portions of the roots.

of the plants showed no abnormal symptoms prior to their final wilting and death. Other plants were stunted and noticeably smaller than normal plants. Affected plants are usually noticed first when they wilt suddenly and die without any preliminary yellowing of the foliage. Plants wilting in this way resemble those dying from *Phymatotrichum* root-rot. However, when plants with strangulated roots are pulled, it is found that instead of the decayed roots characteristic of root-rot, the roots are deformed but sound. Immediately below the surface of the ground, the bases of the stems of typically affected plants are considerably enlarged; below these enlarged portions the taproots taper rapidly to blunt points, which terminate in needle-like, fine rootlets. There are sometimes a few short stubby laterals which also taper to blunt points. The short tap-roots and lateral roots are frequently covered with bead-like calluses, which occur either scattered or in clusters. It is to be noted that this description applies only to the upper portions of the root systems which portions come out readily when the plants are pulled. The deeper parts, which are connected to the upper portions only by the constricted regions described below, remain in the ground and appear normal.

The following measurements were obtained from ten affected plants which were pulled from the field. The plants were large and well-grown, ranging from 22 to 43 cm. tall, with an average height of 32 cm. At the surface of the ground, the diameter of the stems averaged 7.2 mm., while at an average of 4 cm. below ground, the diameter averaged 10.0 mm. The average length of the tapered point (that is, the distance from the thickest part of the root to the blunt tip) was only 2.7 cm. The total distance from the surface of the ground to the bottom of the pointed tap-roots thus ranged from 4 to 10 cm., and averaged just under 7 cm. Only half of the plants had any lateral roots; these averaged less than 2.5 cm. per plant. The typical plants, with very much shortened, wedge-like tap-roots, were almost literally "rootless," with the root systems reduced apparently to less than a twentieth the normal size. There were also less severely malformed plants, with longer and more nearly normal lateral roots. The roots of these plants also were characteristically covered with small bead-like calluses. Plants with roots of this sort (fig. 1 *d*) did not wilt so early in the season as was the case with the plants with more severely affected roots (fig. 1 *b*).

By making field excavations of the root systems of affected plants, it was possible to find the probable cause of the trouble.² Owing to the protracted dry weather during the summer of 1929, the soil around the plants was found to be unusually hard and compact. The method of excavation was

² The writers were assisted by Mr. S. E. WOLFF in excavating plants at the J. W. COFFIN farm at Itasca, Texas.

as follows: Isolated, recently wilted plants were located, and the tops cut off. A deep trench was then dug 12 inches away from the affected plant. With hammer and ice pick, particles of the soil were gently chiseled away until the entire root system of the plant was fully exposed. In some cases, the soil was too dry and hard to enable use of the ice pick. Such plants were removed inside large blocks of soil, which were placed in a wash-tub filled with water. After the hard soil softened, it was then possible to remove the soil from the roots.

It was found that all affected plants, excavated from various fields, actually possessed well-developed root systems. The upper parts of the tap-roots or laterals were, however, separated from the lower parts by constricted areas, often an inch in length, which varied from 0.3 to 0.4 mm. in thickness (fig. 1 *e*). The constricted areas were found in the hardest, subsurface layers of soil; and the more normal lower parts of the roots were found in the less compact soil beneath. It was these needle-like, constricted, connecting portions of the roots which had at first been considered fine rootlets.

Probable cause of cotton root strangulation

This malformation of cotton roots is evidently non-parasitic. There was no evidence of infection or decay in the tissues of the roots, and cultures made from various parts of the roots were uniformly sterile. There was also not the least evidence of injury by implements or insects. It thus appears of significance that the trouble has been found only on sticky, poorly-drained, flat clay soils, and that it occurred only during seasons in which exceptionally heavy rainfall in spring or early summer was followed by prolonged hot, dry weather. Such a sequence of weather conditions occurred both in 1923 and in 1929. In fig. 2, precipitation data are summarized for these years, for one of the fields in which cotton strangulation occurred both years. It will be noted that in 1923, after excessive rainfall in April, there was a drought for four months. Again in 1929, following the record-breaking rains of May, there was very hot, dry weather in June.

A record for one of the farms in which plants were excavated will illustrate the effect of the peculiar weather conditions during 1929 on the cotton crop. The soil was Houston black clay, a deep clay soil which packs and becomes very sticky during rainy weather. Cotton was planted about April 30, and the soil was then thought to be in a fair condition of tilth. By May 4 and 5, the seed came up to a good stand. Frequent beating showers on May 6 and 7 packed the surface of the ground, which was too wet to permit cultivation to break the surface crust, and the rains continued from May 12 to 28. The ground was now thoroughly packed and too soaked for cultivation until June 2. It was not until this time that the ground ap-

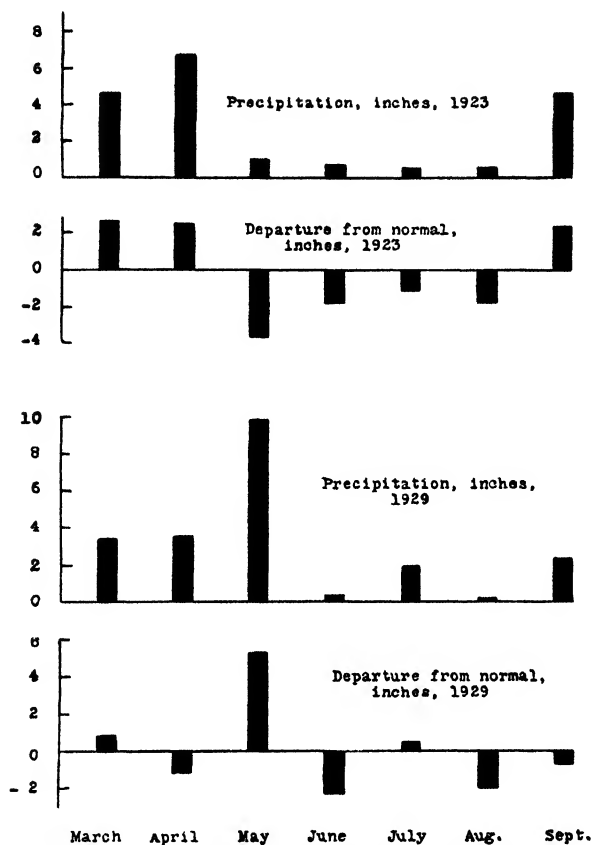


FIG. 2. Rainfall data, 1923 and 1929, averaged from records for Cameron and Valley Junction, Texas, the weather stations nearest to Eloise where root strangulation occurred both years.

peared dry enough for the first cultivation of the season. On account of the hard, packed condition of the soil, the cultivator in many places failed to break the crust. The weather continued hot and dry, and the soil stayed hard and dry, from then until August 17, when affected plants were collected in this field. Plants began to die from root strangulation about the middle of July, and about 5 per cent. had succumbed by August 17.

The following explanation of cotton strangulation thus appears probable. First the seedlings, germinating in the moist soil, developed more or less normal root systems. The early, continued rains packed the surface soil, which was then gradually baked during the hot, dry weather following. The upper portions of the tap-roots and laterals were imprisoned in the hardened, bricklike subsurface layer of soil, and further growth of these

portions of the roots was prevented. Below these imprisoned areas, the roots continued to develop fairly normally. So long as the strangulated areas remained sound, water and food material could still be translocated between roots and stems, although in increasingly inadequate amounts as the plants increased in size. The enlarged, clavate bases of the stems were evidently caused by accumulation of food materials which could not be translocated down to the roots. Final wilting and death of the plants appeared to be directly from lack of water. This may have resulted from inability of the slender connecting tissue to supply sufficient water for unusually hot days, or from death of this thin connecting root in the hard, baked, subsurface soil.

Summary

Root strangulation of cotton has been found in Texas, Arkansas and Mississippi. It seems to occur only in flat, poorly-drained, heavy clay soils, which are compacted by continuous rain or irrigation, and then further hardened, in the absence of cultivation, by continued hot, dry weather. The direct cause of the trouble is apparently that the upper portions of tap-roots and laterals of the young seedlings are caught early in the season in a subsurface layer of hard, dry clay in which further development is prevented. Affected plants die when the constricted areas in the hot, dry soil are killed, or when the moisture supply which can be transported through the constricted areas becomes too greatly inadequate for the requirements of the plants.

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TESTS OF CHIBNALL'S METHOD OF EXTRACTION FOR INVESTIGATING WINTER HARDINESS OF PLANTS¹

W. E. TOTTINGHAM, R. G. SHANDS AND E. D. DELWICHE²

(WITH TEN FIGURES)

CHIBNALL has published (2) a method for extracting separately the vacuolar sap and cytoplasmic fluid in plant cells. It appeared that this procedure might be useful for chemical examination of plant tissues in relation to winter hardiness. The writers were particularly interested in a comparison of the distribution of soluble forms of nitrogen compounds and sugars between the sap and cytoplasmic fluids with the progress of cold weather.

Plant material

A cold resistant variety of wheat was used, varying its resistance by change in the date of sowing. This was Wisconsin Pedigree 2, a strain of the Turkey Red variety. Alfalfa plants were secured from field sowings one year old. A range of hardiness was covered including Grimm as the typically hardy variety, Utah as semi-hardy and Peruvian as non-hardy. Agronomists recognize that winter hardiness of wheat becomes localized in the crown, or base of the stems. In the present investigation the roots were severed and the tissue below the base of the lowest leaf blade taken for analysis. Obviously, this is likely to involve considerable proportions of tissue of lesser pertinence to the investigation, but practical considerations as to the quantity of tissue rendered the grosser sampling necessary. In alfalfa the crown tissue is more limited and less definitely defined than with wheat. Hence, for the present purpose, a top portion of the tap root about 10 inches in length was taken. This was divided into upper and lower sections of about equal length. When the soil became deeply frozen it was necessary to blast, allowing the fragments to thaw at room temperature over night for recovering the plants. It must be admitted that the time elapsed in recovering tissue in this manner might permit considerable changes to occur in its composition. In the earlier samplings of wheat it was found possible to loosen the crowns quickly by playing tepid water over the soil blocks, but the heater available lacked sufficient capacity.

¹ Published with permission of the Director of the Wisconsin Agricultural Experiment Station.

² The writers are indebted to Professors J. G. DICKSON, Department of Plant Pathology, and L. F. GRABER, Department of Agronomy, for support which made this work possible.

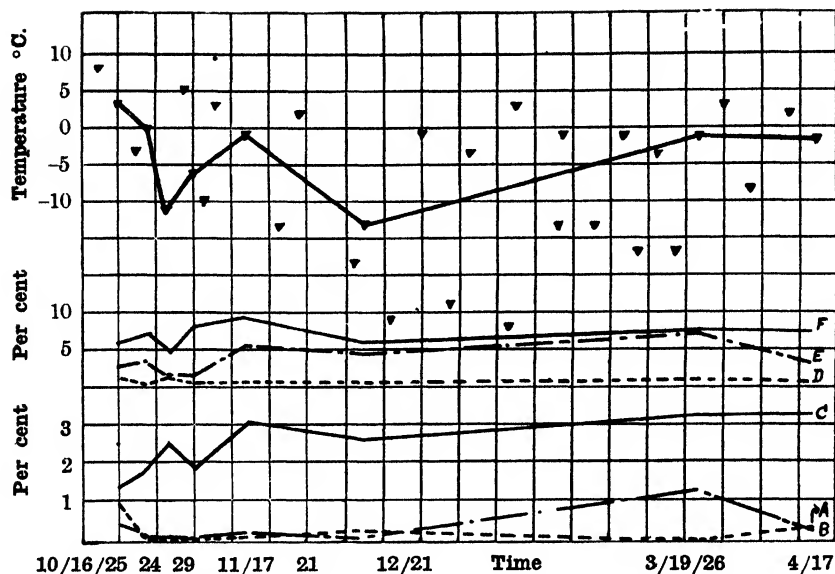


FIG. 1. Relation of sap composition in wheat crown to hardening temperatures. Favorable sowing date. A = protein, B = basic N x 5, C = rest N x 5, D = pentosan, E = reducing sugars, F = sucrose.

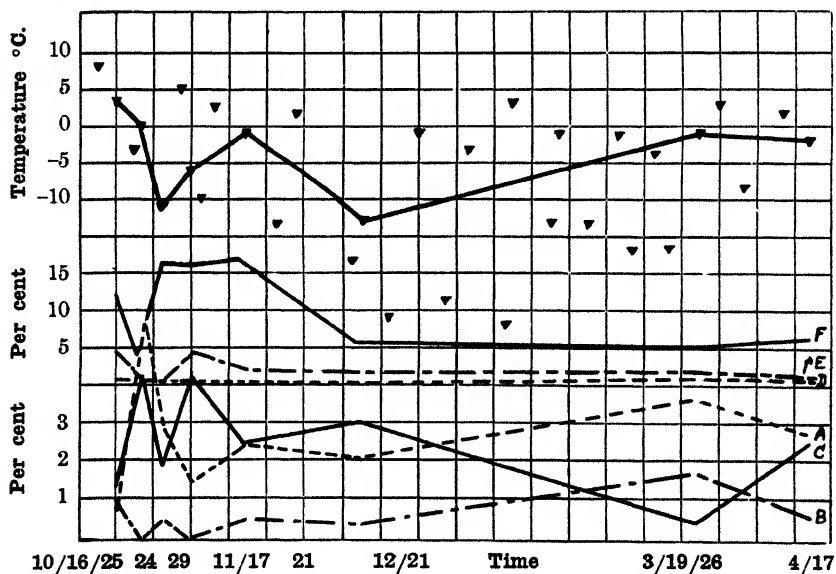


FIG. 2. Relation of extract composition in wheat crown to hardening temperatures. Favorable sowing date. A = protein, B = basic N x 5, C = rest N x 5, D = pentosan, E = reducing sugars, F = sucrose.

Analytical procedure

Inasmuch as this is a test of extractive method, rather than a finished metabolic investigation, it seems sufficient to give here only the broader and more pertinent aspects of both the analytical treatment and the resultant data.

The rapidly washed tissue was freed of surface moisture by absorption and chopped to small fragments. In addition to samples for moisture and total nitrogen, a relatively large aliquot (50 to 100 gms.) was taken for extraction. This was inclosed with a little ether for cytolyzing, expressed in a heavy, hand-operated press, and rinsed out with water to remove the vacuolar sap. The residual tissue was ground in a Nixtamal mill with liberal addition of water, pressed and rinsed in cheese cloth and filtered through a layer of paper pulp. In this way the protoplasmic extract was obtained. Care was taken, of course, to maintain uniform conditions throughout the extraction of successive samples. In the analytical examination procedures conformed to recommendations already published (1). The results are expressed as percentages of the original dry matter.

Obviously, considerable variation in the analytical data should be expected and only those of major magnitude should be seriously considered. For this reason the results are not given in detail but rather in the generalized form of graphs. On these latter the inverted heat symbols show the frequency and extent of major departures in the minimal temperatures. This practice is based upon HARVEY's observation (5) of hardening efficiency in alternating temperatures.

Interest in the results here presented is conditioned by the possibility that proteins of the protoplasm may either enter into combination with other compounds protective against cold or become favorably altered in the hardening process. Such changes should probably be anticipated less in the sap, but it should be recognized that exchanges of constituents may occur in both directions between the cytoplasm and vacuolar sap.

Discussion

The results for wheat from a date of planting favorable to cold hardiness are depicted in figures 1 to 3, inclusive. In this case the sap protein exhibits marked depression throughout the period of sub-zero temperatures. This loss was compensated by an increase of other than basic nitrogen compounds, which may be assumed with considerable justification to consist largely of simpler peptides and monamino-acids. These changes were accompanied by increases of sugars, and particularly of sucrose, in the early period of lower temperatures.

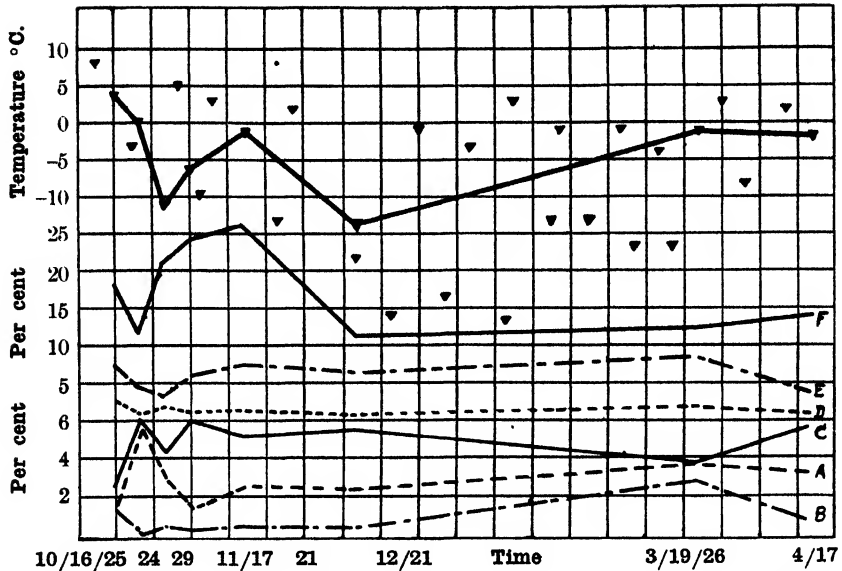


FIG. 3. Relation of total solutes composition in wheat crown to hardening temperatures. Favorable sowing date. A = protein, B = basic N x 5, C = rest N x 5, D = pentosan, E = reducing sugars, F = sucrose.

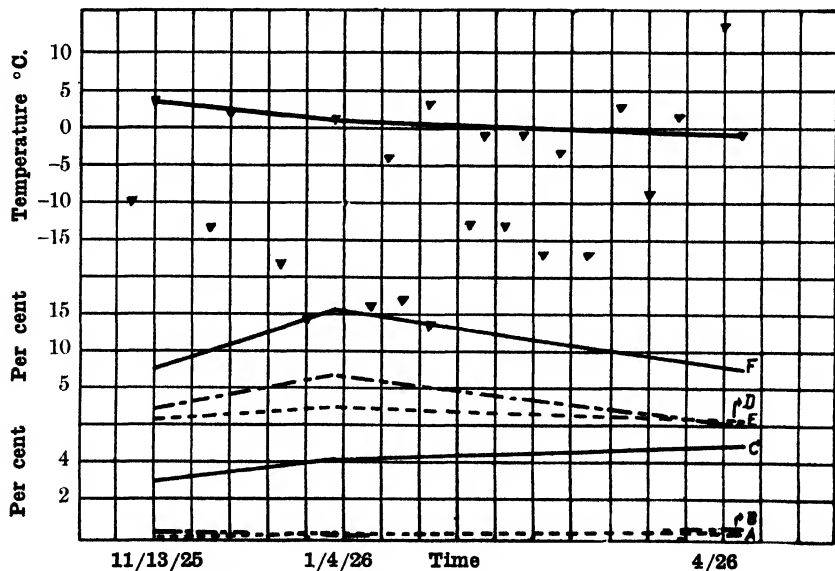


FIG. 4. Relation of sap composition in wheat crown to hardening temperatures. Unfavorable sowing date. A = protein, B = basic N x 5, C = rest N x 5, D = pentosan, E = reducing sugars, F = sucrose.

The protein of the extract appears to have varied sharply, but its general trend was toward increasing with hardiness. However, this relation applies more distinctly to the "rest" fraction, already postulated as involving simpler peptides and monamino-acids. Concomitant with these changes was a marked accumulation of sucrose. This phenomenon is particularly striking. Apparently sucrose was fixed in some manner in the protoplasm or it should be more largely recovered in the sap. This furnishes evidence additional to that of NEWTON (7) of protection provided by sucrose against coagulation of protein by freezing. The present indications of hydrolysis of proteins in hardening is in agreement with the early observation of HARVEY (4) on cabbage and that of NEWTON (7, p. 39) on wheat. No further interpretation is suggested by the combined values of sap and extract.

The results with wheat from the later, unfavorable date of sowing are shown in figures 4 to 6, inclusive. Here the sap displays a continuous increase of the "rest" nitrogen fraction. This change was accompanied by increases of all forms of carbohydrates during the hardening season, sucrose predominating. In the extract the variation of nitrogen compounds was relatively insignificant during the earlier cold weather. During this period a decided increase of reducing sugars was accompanied by a striking decrease of sucrose. These latter changes are conspicuous in the summated results, and are in marked contrast to those occurring in the test with hardier plants.

In the case of alfalfa roots the results are presented in figures 7 to 10, inclusive. The sap shows little orderly change in nitrogen compounds excepting a late increase of the alpha-amino fraction in the Grimm variety. Inspection of the temperature graph indicates that one might not expect hardening to have been far advanced until the last date of sampling. Sugars increased a little in the order of hardiness. In the extract, protein increased appreciably in Grimm but not in the less hardy varieties. A similar trend is shown by the alpha-amino fraction. The sugar curves give no consistent variation but those of the dextrin-starch fraction show declines in the order of hardiness. No further observations are suggested by the summated composition of sap and extract.

Of the insoluble components the variations of protein are too irregular to bear interpretation. The values of this constituent were based on the assumption that all nitrogen remaining in the tissues after extraction was present in proteins. To it is added the protein of sap and extract to give total protein. Decrease of starch in all varieties is one of the most definite effects here observed, being most prominent in Grimm and at an early stage. These observations of increases in sugars and soluble nitrogen compounds

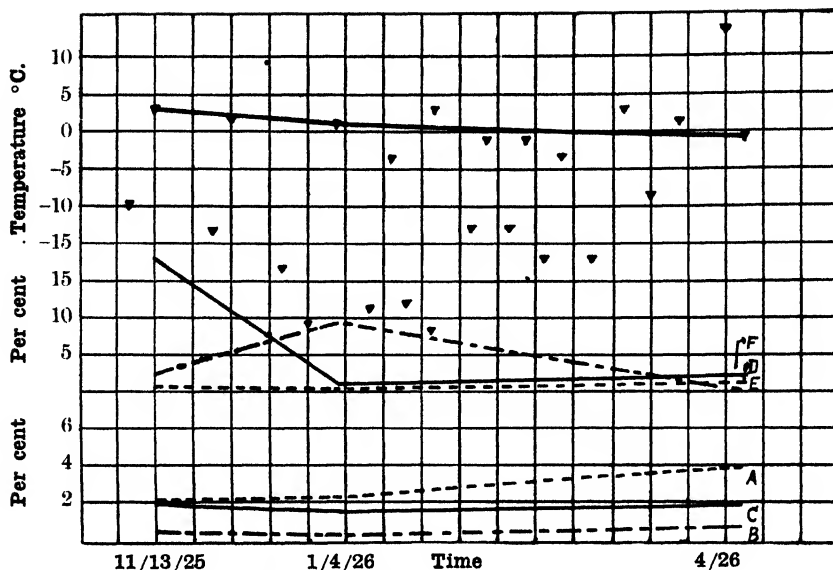


FIG. 5. Relation of extract composition in wheat crown to hardening temperatures. Unfavorable sowing date. A = protein, B = basic N x 5, C = rest N x 5, D = pentosan, E = reducing sugars, F = sucrose.

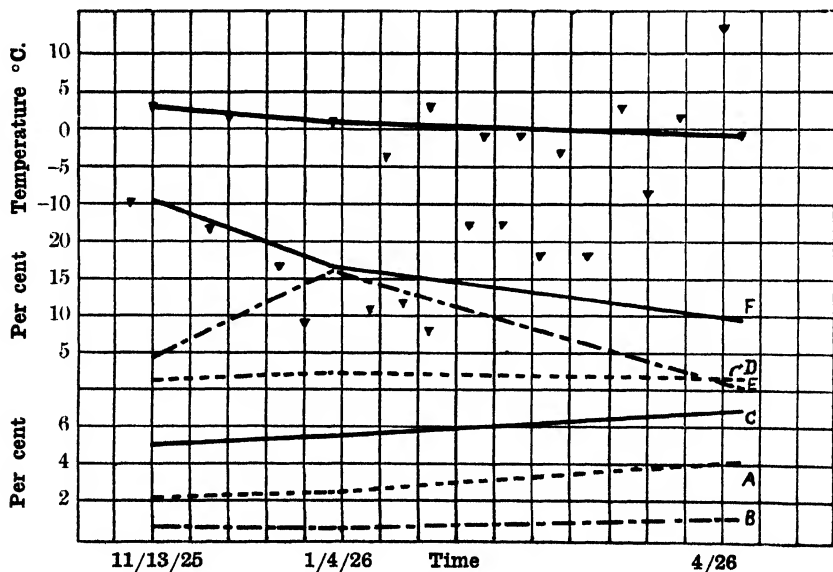


FIG. 6. Relation of total solutes composition in wheat crown to hardening temperatures. Unfavorable sowing date. A = protein, B = basic N x 5, C = rest N x 5, D = pentosan, E = reducing sugars, F = sucrose.

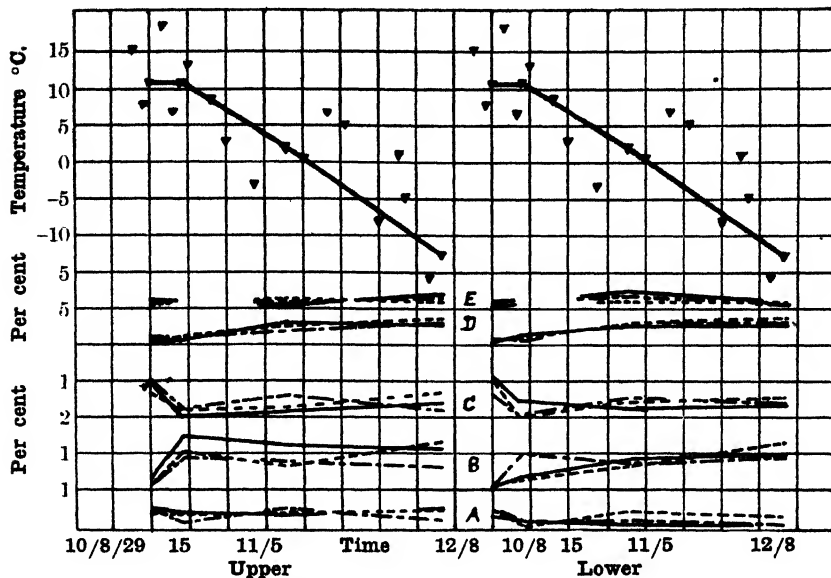


FIG. 7. Relation of sap composition to hardening in alfalfa root. ---- = Grimm, — = Peruvian, - · - = Utah. A = protein, B = α -amino N \times 5, C = rest N \times 5, D = total sugars, E = dextrins and soluble starches.

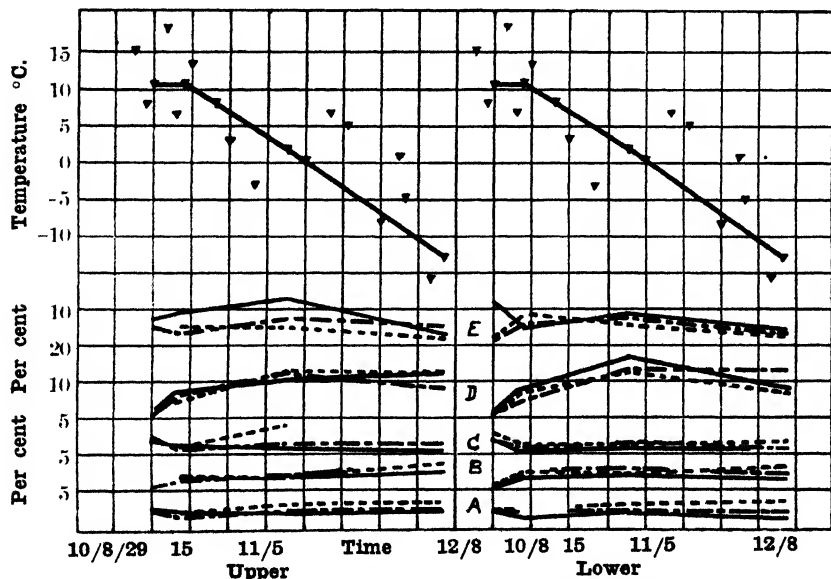


FIG. 8. Relation of extract composition to hardening in alfalfa root. ---- = Grimm, — = Peruvian, - · - = Utah. A = protein, B = α -amino N \times 5, C = rest N \times 5, D = total sugars, E = dextrins and soluble starches.

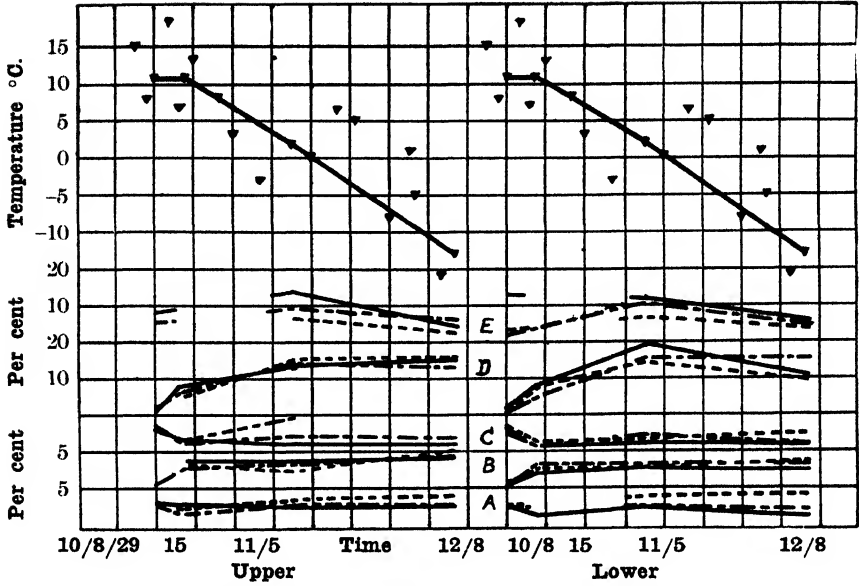


FIG. 9. Relation of total solutes composition to hardening in alfalfa root. ---- = Grimm, — = Peruvian, - - - - = Utah. A = protein, B = α -amino N x 5, C = rest N x 5, D = total sugars, E = dextrans and soluble starches.

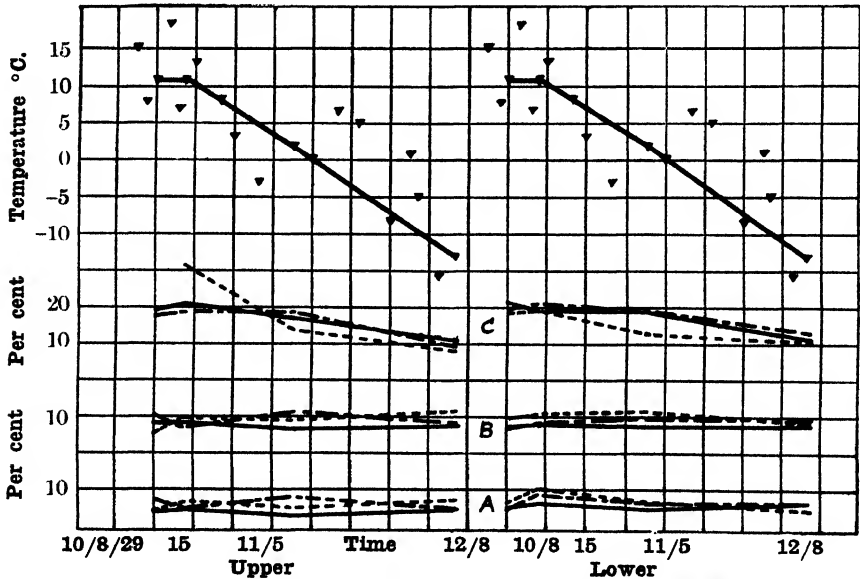


FIG. 10. Relation of insoluble constituents to hardening in alfalfa root. ---- = Grimm, — = Peruvian, - - - - = Utah. A = protein, B = total protein, C = starch.

and decrease of starch agree with the results of STEINMETZ (8) and JANSSEN (6).

Some constituents, such as extract protein and insoluble starch, display good agreement between values of upper and lower root. This is not to be expected generally, for the bud and crown tissue included in the upper section might well differentiate its metabolic behavior from that of other root tissue. Numerous irregularities in the data indicate that extensive operations might be necessary according to the procedures here followed to yield conclusive results. If sampling could be localized to the more pertinent crown tissues, as suggested by the observations of STEINMETZ (8, p. 10), one might hope for more significant results. Because of our immediate interest in a method of attack upon the hardness problem developed at this institution (3) it is unlikely that we can soon give CHIBNALL's method the further tests it appears to merit. We therefore present the results of these limited trials on their face value.

Conclusions

Separate chemical analysis of the protoplasmic extract by CHIBNALL's method of recovery from selected tissues of wheat and alfalfa discloses changes of composition which may be related to the development of cold hardness. This method of tissue fractionation appears to merit further tests of its usefulness.

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COMPOSITION AND QUALITY OF PENNSYLVANIA CIGAR-LEAF TOBACCO AS RELATED TO FERTILIZER TREATMENT^{1, 2}

D. E. HALEY, J. B. LONGENECKER AND OTTO OLSON

The quality of the smoke produced during the normal combustion of a cigar is largely dependent on the chemical composition of the tobacco. The chemical composition of the tobacco may be influenced by many factors, among which is the fertilizer treatment employed by the grower. In order to obtain the best quality cigar-leaf tobacco, it is essential to recognize the requirements and limitations of the growing plant, to insure the presence of the materials deemed necessary, and to guard against those materials that are not only unnecessary but even harmful, so far as quality production is concerned. The individual effects of a fertilizer treatment, however, may be considerably influenced by many environmental conditions, among which is the inherent fertility of the soil itself. For example, the soil of the experimental tobacco plots at Ephrata, Lancaster County, Pennsylvania is extremely high in calcium and quite low in available potassium. This may not hold true for tobacco soils in other sections of the county; hence the optimum fertilizer treatment for the experimental plots may not hold true for other soils in the county.

In former publications (1, 2) we have recognized the fact that for quality production of tobacco on our experimental plots we must equalize, if possible, the calcium and potassium content of the plants. In 1928 we planned an experiment having this objective.

Plan of the experiment

Avoiding the use of lime, but not taking into account those factors which might influence the availability of soil calcium, we planned an experiment making potash the most important variable. Ten plots were used, each one-thirtieth of an acre in area. The crop previously grown on these plots was red clover. Five of the plots received manure, with or without additions of fertilizer. The remainder received individual fertilizer treatments, but no manure. The treatments are given in table I.

At the end of the growing season twenty plants from each plot were air-cured. At the end of this period they were dried at room temperature. When dry, the webs of the leaves were ground and kept in sealed receptacles for analysis.

¹ Publication authorized by the Director of the Pennsylvania Agricultural Experiment Station as Technical Paper no. 505.

² This investigation was conducted in cooperation with Dr. W. W. GARNER, of the U. S. Bureau of Plant Industry, Office of Plant Nutrition and Tobacco Investigations, and Professor F. D. GARDNER, Department of Agronomy of the Pennsylvania State College.

TABLE I
FERTILIZER TREATMENTS OF THE EXPERIMENTAL PLOTS
ACRE BASIS

PLOT NO.	MANURE	COTTON-SEED MEAL	PRECIPITATED BONE* PHOSPHATE	NITRATE OF SODA	SULPHATE OF POTASH	CARBONATE OF POTASH	UREA
	<i>tons</i>	<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>
A-1	10	400	130	125	200	.	.
A-2	10	400	130	125	400	.	.
A-3	10	400	130	125	600	.	.
A-4	10	400	130	125		310	.
A-5	10						.
A-6		600	195	187	300		.
A-7		600	195	187	600		.
A-8		600	195	187	900		.
A-9		600	195	187		465	.
A-10		375	460	187		465	57

* 36 per cent. P_2O_5 .

The remaining plants were similarly cured; at the end of this period they were fermented for several months. Uniform samples then were taken for analyses, as mentioned above, and a part of the remainder were made into cigars.

Analyses of the samples were made according to methods as described in previous publications (1, 2), with but few modifications. A special effort, however, was made to determine separately the soluble and insoluble portions of the total ash, thus guarding against the possibility of weighing the calcium as the hydrate or carbonate rather than the oxide.

TABLE II
STUDY OF THE CALCIUM, MAGNESIUM, SULPHUR AND POTASSIUM CONTENT OF CURED TOBACCO
AS INFLUENCED BY FERTILIZER TREATMENT

PLOT NO.	CaO	MgO	SO ₃	K ₂ O
	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>
A-1	7.95	0.84	1.14	2.57
A-2	7.55	0.75	1.16	3.16
A-3	7.45	0.80	1.32	3.35
A-4	7.76	0.81	1.16	3.21
A-5	7.65	0.96	1.18	1.74
A-6	8.09	0.92	1.40	2.81
A-7	6.97	0.82	1.47	3.26
A-8	7.01	0.85	1.68	3.27
A-9	7.23	0.81	1.05	2.84
A-10	7.67	0.85	1.49	2.23

Analytical results

A study of the calcium, magnesium, sulphur, and potassium of the cured samples was made first; the results are given in table II.

This analysis was followed by a study of the soluble and insoluble ash constituents of both the cured and fermented samples, and their alkalinity; the results are given in tables III and IV.

TABLE III

ASH OF THE CURED AND FERMENTED CIGAR-FILLER TOBACCO AS MODIFIED BY FERTILIZER TREATMENT

PLOT NO.	ASH					
	SOLUBLE		INSOLUBLE		TOTAL	
	CURED	FERMENTED	CURED	FERMENTED	CURED	FERMENTED
	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>
A-1	3.99	5.49	13.47	10.45	17.46	15.94
A-2	4.64	6.01	11.95	10.63	16.59	16.64
A-3	4.76	6.38	12.02	10.10	16.78	16.48
A-4	4.51	5.70	11.89	10.61	16.40	16.31
A-5	2.79	3.77	12.85	10.28	15.64	14.05
A-6	3.57	5.40	12.33	10.67	15.90	16.07
A-7	5.00	4.90	11.48	9.94	16.48	14.84
A-8	5.13		12.74		17.87	
A-9	4.23	4.97	12.71	10.53	16.94	15.50
A-10	3.44	5.02	12.74	10.57	16.18	15.59

TABLE IV

ALKALINITY OF THE ASH OF CURED AND FERMENTED TOBACCO

PLOT NO.	ALKALINITY OF ASH					
	SOLUBLE		INSOLUBLE		TOTAL	
	CURED	FERMENTED	CURED	FERMENTED	CURED	FERMENTED
	<i>cc.</i>	<i>cc.</i>	<i>cc.</i>	<i>cc.</i>	<i>cc.</i>	<i>cc.</i>
A-1	46.7	43.6	309.6	318.7	356.3	362.3
A-2	45.7	48.3	286.5	310.6	332.5	358.9
A-3	36.6	47.0	292.5	300.5	329.1	347.5
A-4	38.5	49.6	287.0	312.6	325.5	362.2
A-5	28.1	28.2	287.0	307.9	315.1	336.1
A-6	25.1	40.2	332.4	335.4	357.5	375.6
A-7	37.3	30.2	296.0	293.2	333.3	323.4
A-8*	30.1		300.0		330.1	
A-9	35.4	41.9	309.1	305.9	344.5	347.8
A-10	31.3	37.5	332.3	317.0	363.6	354.5

* The sample of fermented material from this plot was not received.

The nitrogen-carbon ratio of the cured and fermented samples then was ascertained (table V) and the results of smoking the experimental cigars are given in table VI.

TABLE V
NITROGEN-CARBON RATIO OF CURED AND FERMENTED TOBACCO

PLOT NO.	CARBON		NITROGEN		N-C RATIO	
	CURED	FERMENTED	CURED	FERMENTED	CURED	FERMENTED
	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>		
A-1	40.25	40.74	4.42	5.17	1: 9.1	1: 7.9
A-2	42.06	40.01	4.38	5.00	1: 9.6	1: 8.2
A-3	40.96	41.16	4.29	4.81	1: 9.5	1: 8.6
A-4	42.05	41.62	4.80	4.71	1: 8.8	1: 8.8
A-5	41.86	41.17	4.47	4.95	1: 9.4	1: 8.3
A-6	40.92	41.07	4.51	4.93	1: 9.1	1: 8.3
A-7	40.95	41.56	4.57	5.14	1: 9.0	1: 8.1
A-8	42.11		4.44		1: 9.5	
A-9	40.07	41.36	4.51	5.18	1: 8.9	1: 8.0
A-10	42.24	42.03	4.24	4.45	1: 10	1: 9.4

Discussion of results

Analyses of the cured samples showed that it was not possible to materially decrease the calcium or increase the potassium content by the fertilizer treatments employed; this is shown in table II. Analyses of the fermented samples, however, as shown in table III, indicate a more narrow potassium-calcium ratio. According to table II, the sulphur content was not influenced by the large quantity of sulphate of potash additions. These results indicate that the solution of the problem may not be reached by potash applications alone. More attention must be given to those factors which have to do with the availability of soil calcium. In this connection the advisability of plowing under a crop of clover, which is a heavy calcium feeder and maintains a high nitrogen content of the soil, is questioned.

The data in table III show a marked decrease in soluble ash of the fermented, as compared with the cured samples. Probably this is due to the loss of such material in the handling of the tobacco. Considerable quantities of soil and sand may adhere to the leaves of the harvested plants, owing to a gummy covering of the leaves, and even may persist over the period of air-curing. As this gummy material disappears during fermentation, there is a chance for a noticeable loss of inorganic material in this process. At the same time, there is a possibility that an equilibrium is established between the web and the midrib or stem during fermentation, resulting in a loss of calcium from the web to the midrib or stem and an increase of substances, such as potassium, in the web at the expense of these portions.

TABLE VI
SMOKING QUALITIES OF CIGARS MADE FROM THE 1928 CROP
THREE-YEAR ROTATION*

SAMPLE NO.	BURN	FIRE-HOLDING CAPACITY	COLOR OF ASH	COHERENCE OF ASH	AROMA	TASTE	TOTAL**
	<i>points</i>	<i>points</i>	<i>points</i>	<i>points</i>	<i>points</i>	<i>points</i>	<i>points</i>
A-1	18	10	8	8	15	15	74
A-2	17	10	8	6	18	15	74
A-3	18	10	6	8	18	15	75
A-4	19	10	8	8	18	15	78
A-5	15	5	8	6	10	12	56
A-6	18	10	6	8	16	14	72
A-7	18	10	6	8	16	14	72
A-8	18	10	8	8	18	16	78
A-9	16	7	6	8	16	14	67
A-10	16	10	5	8	16	14	69

- * A-1 Coherent. Light gray ash. Slight char. Holds fire 6-7 minutes.
 A-2 Flakes slightly. Light ash. Slight char. Holds fire 6-7 minutes.
 A-3 Coherent. Medium gray ash. Slight char. Holds fire 6-7 minutes.
 A-4 Coherent. Light ash. Slight char. Holds fire 6-7 minutes.
 A-5 Fairly coherent. Mottled ash. Chars badly. Holds fire less than 5 minutes.
 A-6 Coherent. Medium gray ash. Chars slightly. Holds fire 6-7 minutes.
 A-7 Coherent. Medium gray ash. Chars slightly. Holds fire 6-7 minutes.
 A-8 Coherent. Light gray ash. Chars very slightly. Holds fire 6-7 minutes.
 A-9 Coherent. Medium gray ash. Chars. Holds fire 5-6 minutes.
 A-10 Coherent. Dark ash. Chars. Holds fire 6-7 minutes.

** A perfect score would have been as follows:

Burn	20 points
Fire-holding capacity	10 points
Color of ash	10 points
Coherence of ash	10 points
Aroma	25 points
Taste	25 points

There has been a material increase, in most instances, in the soluble ash of the web during fermentation; these differences in percentages cannot be accounted for on the basis of a loss of organic matter during fermentation. If this is correct, then the fermentation of cigar-leaf tobacco has a significance not usually taken into consideration. In order to correctly interpret the data in tables III and IV, however, the total loss of ash constituents and organic matter should be known.

The data in table V show, with one exception, a narrowing of the nitrogen-carbon ratio in the fermentation process. They further show that there must have been a relatively small amount of nitrogen lost during the fer-

mentation process. It must be remembered, however, that the fermentation was not sufficiently prolonged to insure the best quality of tobacco. The carbon content of the cured and fermented samples is, on the whole, quite uniform.

Table VI shows that cigars made from the tobacco of plot A-5 were inferior to the others. On the whole, however, the smoking tests were unsatisfactory. We believe that the lack of sufficient fermentation and aging were of overshadowing importance. All cigars tested showed a relatively high chlorophyll content.* In order to study the effect of fertilizer treatments on tobacco by the score-card system employed, it is desirable that the tobacco be fermented and aged for a much longer period of time before being made into cigars for testing.

Conclusions

The results of these investigations appear to warrant the following conclusions:

1. The fertilizer treatments employed did not materially alter the ratio of potassium to calcium in the cured leaves.
2. Increasing the quantity of sulphate of potash in the fertilizer treatments does not result in increasing the quantity of sulphur in the leaves.
3. During the process of fermentation there is an apparent increase of soluble ash constituents in the leaves.
4. There is a narrowing of the nitrogen-carbon ratio during fermentation, but very little, if any, loss of nitrogen.
5. The effect of the fertilizer treatment on the burning qualities of the cigars made from the differently treated tobaccos, is overshadowed by insufficient fermentation and aging. Qualitative tests of these cigars showed a relatively high concentration of chlorophyll, which is not only undesirable itself, but also indicates insufficient fermentation.

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* Chlorophyll, however, may be found in many of the cigars now on the market.

A STUDY OF THE AMMONIA CONTENT OF CIGAR SMOKE^{1, 2}

D. E. HALEY, C. O. JENSEN AND OTTO OLSON

(WITH ONE FIGURE)

The number of cigars manufactured in the United States for the year 1913 exceeded 8,500,000,000, while in 1928 the number produced was about 7,000,000,000. These figures indicate a noticeable decrease in demand. Probably the chief cause for this is the increasing popularity of cigarettes. However, the quality of the cigars now on the market may be a factor of considerable importance. The curing, fermentation, and aging processes have a great deal to do with the development of a pleasant aroma, lack of harshness, and a mild physiological effect when smoked, but the field treatment of the tobacco itself may be of overshadowing importance.

Pennsylvania ranks high as a producer of cigar-leaf tobacco. The Pennsylvania Agricultural Experiment Station and the United States Department of Agriculture have been interested for a number of years in the factors that affect quality production. Various field treatments have been employed at the Tobacco Experiment Plots at Ephrata. Each year a considerable quantity of the tobacco produced is fermented and made into cigars for testing purposes. As a rule, the cigars are made from tobacco less than a year after the crop is harvested. Because of insufficient aging, these cigars usually produce a harsh unpleasant smoke. The burn, coherence of ash, and other qualities may, however, be studied satisfactorily.

The physiological effect and other undesirable qualities of cigar smoke have been attributed, in large measure, to the nicotine content, although cigar smoke is known to contain ammonia, aldehydes, amines, organic acids, carbon monoxide, hydrocarbons, hydrogen sulphide, hydrogen cyanide, pyridine, and many other substances. From a physiological standpoint, ammonia is an important constituent in that it may interfere with the normal action of the heart and produce other complications if present in the smoke in sufficient concentrations.

Very little has been reported on the ammonia content of cigar smoke, especially for cigars made from tobacco of known history. For this reason it was decided to make a study of this constituent in the smoke of cigars made from the experimental tobaccos.

¹ Publication authorized by the Director of the Pennsylvania Agricultural Experiment Station as Technical Paper no. 506.

² This investigation was conducted in cooperation with Dr. W. W. GARNER, of the U. S. Bureau of Plant Industry, Office of Plant Nutrition and Tobacco Investigations, and Professor F. D. GARDNER, Department of Agronomy of the Pennsylvania State College.

Experimental

The test cigars were made wholly of tobacco grown on 10 separate plots which received fertilizer treatments according to the plan given in a previous paper (5). An intermittent smoking apparatus was used. Somewhat similar methods have been employed by others. JENKINS (6) used an apparatus in which suction was secured by means of an aspirator which filled by a continuous inflow of water and emptied at regular intervals by means of a siphon. GARNER (4) retained the essential features of this apparatus, but modified it so that several cigars could be smoked simultaneously. GARNER's apparatus, however, was devised for work pertaining to the burning qualities of the cigar rather than to the chemistry of the smoke. The duration of each puff was 10 seconds; the interval between puffs was 30 seconds. WILEY (9) describes a similar apparatus. ASHERSON (1) used an aspirator which evidently was turned on and off by hand, so as to simulate the manner of smoking of the average smoker. BOGEN (2) states that he obtained the necessary suction by the use of a water pump which was turned on and off at regular intervals by an electric solenoid valve, operated by a contact on a Harvard kymograph. An automatic siphon arrangement was tried, but was discontinued as unsatisfactory.

Various methods have been employed for collecting the active constituents of smoke. BOGEN (2) reports that he collected the smoke over water, allowed it to condense for one hour and then analyzed the aqueous solution for ammonia. THOMS (7) employed three jars containing various quantities of 10 per cent. H_2SO_4 in order to remove the basic constituents of the smoke.

Methods of analyses employed by other workers in this field were reviewed. VICKERY and PUCHER (8) developed a method for estimating the ammonia in tobacco and tobacco extracts which is entirely satisfactory for work of this kind. It is based on the observation that nicotine is absorbed by permutit (a synthetic aluminosilicate) only to a very small extent, whereas ammonia may be quantitatively removed from a faintly acid solution by permutit, set free by alkali and determined by Nesslerization. The method which is fully described by VICKERY and PUCHER (8) and which is a modification of FOLIN and BELL's method for the determination of ammonia in urine (3), was used by us and found quite satisfactory.

The smoking apparatus

Some of the apparatus previously used by us proved unsatisfactory. It was felt that an intermittent siphon could be made that would give a regular interval in the suction. An apparatus was devised which proved satisfactory (see fig. 1). It was so regulated that each puff lasted about 6.5 seconds with an interval between puffs of 35 seconds.

A is a suction flask to which continuous suction is applied by means of a laboratory vacuum pump. The amount of suction is regulated by means of a valve. *B*, *C*, and *D* are absorption tubes, each of which holds 25 cc. of 20 per cent. H_2SO_4 . *E* is a glass cigar holder. *F* is a tube admitting air to the suction flask *A*, when the water level in the intermittent siphon *G* is below the level of the inverted tube *F*. When the water rises to the level of the inverted funnel, the air supply is cut off and the vacuum created in *A* draws air through the cigar. When the water reaches the top of the curved tube in *G*, it siphons out and the tube *F* is again open, air enters the suction flask *A* and no air is drawn through the cigar. *I* is a bottle holding water at a constant level, and fed from the supply bottle *J*. The rate of flow of water from *I* is regulated by means of the clamp *H*.

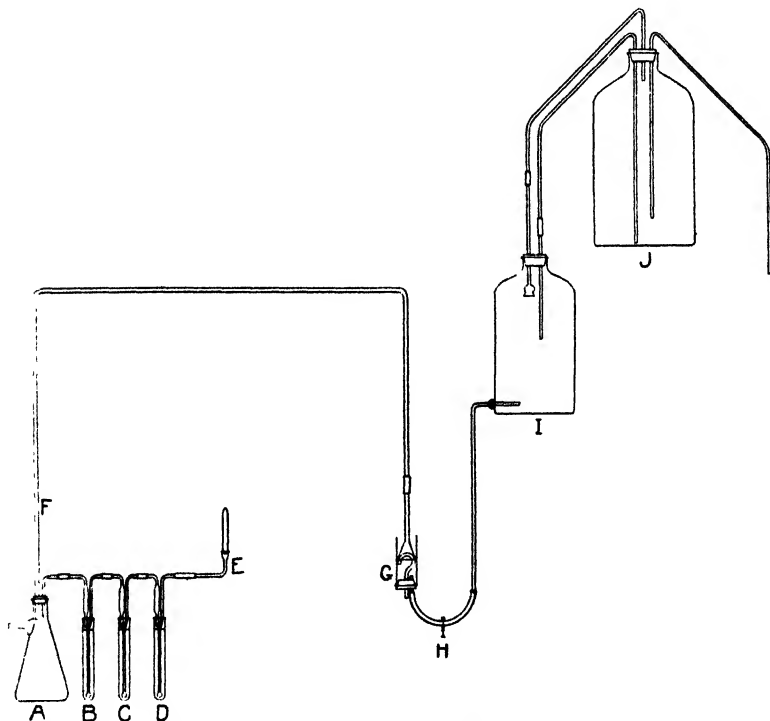


FIG. 1. Intermittent smoking apparatus.

Before smoking, the cigars were kept in a desiccator containing 43 per cent. H_2SO_4 . At 25°C ., according to WILSON (10), this should give an atmosphere having a relative humidity of 50 per cent. The ends of the cigars were cut so that all had the same circumference. Each cigar then was weighed and smoked. The small quantity of tobacco remaining at the

end of the experiment was weighed and subtracted from the original weight. In this way the weight of tobacco smoked was ascertained.

In the preliminary work it was found that 25 cc. of normal H_2SO_4 in the first tube absorbed practically all of the ammonia. Since we desired to determine nicotine, however, we used 20 per cent. H_2SO_4 instead.

Three cigars were smoked before the acid was removed. The total amount of ammonia and its relation to the total nitrogen content of the tobacco then was determined. The results are given in table I.

TABLE I

QUANTITY OF AMMONIA IN SMOKE AS RELATED TO THE TOTAL NITROGEN CONTENT OF THE ORIGINAL TOBACCO FROM WHICH THE CIGARS WERE MADE

CONSTITUENTS	SAMPLES									
	A-1	A-2	A-3	A-4	A-5	A-6	A-7	A-8	A-9	A-10
	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
Total nitrogen	51.7	50.0	48.1	47.1	49.5	49.3	51.4		51.8	44.5
Ammonia in smoke	4.7	5.4	5.5	5.6	5.4	5.4	6.0	4.8	3.5	3.6

The results show that apparently there is no relation between the ammonia content of cigar smoke and the fertilizer treatment received by the tobacco. This is not strange since the fertilizer treatment did not materially affect the nitrogen content of the tobacco. The first seven samples show a close correlation between the total nitrogen content of the tobacco and the quantity of ammonia in the smoke. Representative samples of several commercial cigars made almost wholly of well fermented tobacco showed a smoke of much lower ammonia content.

Summary

1. An apparatus was devised which proved satisfactory for the intermittent smoking of cigars.
2. There was no correlation between the fertilizer treatment of the tobacco and the ammonia content of the smoke. This may not hold true for cigars made of thoroughly fermented tobacco.

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JEAN SENEBIER
1742-1808

BRIEF PAPERS

JEAN SENEBIER

1742-1808

(WITH ONE PLATE)

“Mais la meilleure de toutes les méthodes pour assurer la bonté de ses observations; c'est celle par laquelle on cherche à les appuyer sur de nouvelles observations faites en sens contraire: l'opposition des résultats démontre alors la justesse des premières observations.”

L'Art d'Observer, I, 209.

CALANDRINI (1743) first expressed the surmise that the leaves of plants possessed the function of collecting and absorbing dew. This induced a wealthy patrician in Geneva, CHARLES BONNET (1720-1793), to immerse shoots of a grape vine in large glass vessels. He at once observed that these shoots would be covered with innumerable air bubbles as long as sunlight lasted. After sunset, the phenomenon ceased.

This observation, which dates from 1747, opened the door to thousands of experiments at the hand of BONNET's younger contemporaries: PRIESTLEY, INGEN-HOUSZ, SENEBIER, and DE SAUSSURE. By cumulated efforts these four investigators, aided by the analytical work of LAVOISIER, succeeded in disclosing the essentials of that mystery which a later period named the phenomenon of photosynthesis, or the discovery of carbonic acid as the chief element of plant nutrition.

By association and inclination SENEBIER was predisposed to a studious life. The son of a wealthy merchant, he was born in Geneva, in 1742 and might have entered commerce, but preferred a free activity as a student of science. His family consented, except for the stipulation that the young man must take up a definite study and finish it. He chose theology and was admitted to the pastorate after three years. At an early age he had been attracted to the circle inspired and guided by BONNET, who fired his younger contemporaries by that curiosity which led to experiments rather than contemplation and philosophy.

After his graduation SENEBIER made a journey to Paris where, on BONNET's advice, he competed for a prize announced by the Haarlem academy for the best answer to the question “Wherein consists the art of making observations?” He won the prize, and returned to Switzerland where, in 1769, he became pastor at Chancy and remained in the pastorate until 1783, when he was called to Geneva as librarian of the City Library. Several works undertaken by him at this period indicate that his inclinations were divided between pure science and literary history. He translated into French the *Opuscula* of SPALLANZANI; he published a literary history of Geneva and calendared and annotated the manuscripts of the municipal library with great zeal. In 1787, he joined the staff of the

renowned *Encyclopédie méthodique* as collaborator for plant physiology. His personal work in this field was published before the Revolution, when SENEBIER returned to the little town of Rolle, carrying his laboratory equipment with him. He continued his physiological, meteorological and chemical researches there for more than ten years and printed numerous papers in the publications of several learned societies in which he held membership, notably Paris, Turin, Geneva and Lausanne. Returning to Geneva in 1799 he divided his attention between the preparation of a translation of the apocryphal books and his *Physiologie végétale* (1800) and died in 1808.

SENEBIER'S "physical" experiments, historically an inspiring episode in our knowledge of photosynthesis, began as early as 1765, and their progress, which we shall try to follow, went hand in hand with theoretical and methodological contemplation of the highest order. The results of these studies constitute SENEBIER'S first important book: *Essai sur l'art d'observer*, 1775. This book, I believe, is the first systematic attempt at a philosophy of the art of experimentation. The young physicist describes in great detail the mental processes, the points of view, the conscientious attention, and the skill and inventiveness necessary to an experimental worker. Each human quality fully developed, each instance of high inspiration, affords some possibility which insures good and reliable work. In its main thesis, in its appeal to the harmonic personality of the scientific worker, the book still is valid and may be read with advantage even now.

The publications of SENEBIER on plant physiology are quite numerous, but nearly all his experimental work was concentrated in the following books: 1. *Mémoires physico-chimiques sur l'influence de la lumière pour modifier les êtres des trois règnes de la nature et surtout ceux du regne végétal*. 3 vols. Geneva, 1782. 2. *Recherches sur l'influence de la lumière solaire pour métamorphoser l'air fixe en air pur par la végétation*. Geneva, 1783. 3. *Expériences sur l'action de la lumière solaire dans la végétation*. Geneva, 1788. 4. *Physiologie végétale*. 5 vols. Geneva, 1800.

The titles of these works elucidate not only the contents, but, chronologically considered, also the historical development of SENEBIER'S studies of the physical basis of photosynthesis. Scarcely any other similar series of experiments in any field of research contains a similar wealth of detail; hundreds and hundreds of times was the same arrangement repeated, now with reference to INGEN-HOUSZ, then testing the exactness of PRIESTLEY'S work, then again striking a new track.

The fundamental apparatus was similar to that of INGEN-HOUSZ. The objects (leaves of plants) were deposited in a jar of water; above them and immersed in the jar, stood an inverted funnel, the neck of which was closed at its upper end and graded in order that the gas discharged by the vegetable matter might be measured. But one leaf was used in each experiment, and in each instance its surface was measured by means of a "phyllo-

meter," two glass plates, one ruled to squares, between which each leaf was measured by the number of squares which it covered. The "exhaled" gas was tested by means of VOLTA's eudiometer.

In his first book (1779) SENEBIER denied that his plants ever developed carbonic acid under any circumstances. Green plants developed no gas in the dark. He also pointed out that oxygen was developed only by green plants in sunlight; etiolated leaves, flowers and similar structures gave only negative results. The oxygen originated in the chlorophyll-bearing parenchyma, not from leaf ribs or from epidermal structures. Even small fragments of green parenchyma secreted oxygen.

Another series of experiments was concerned with the influence of colored light on the formation of oxygen. The priority of these studies is his. He employed large, double-walled bell-bottles and tested his objects with blue (litmus solution) and yellow (curcuma extract) light. The results indicated abnormal relations, but these were considered due to the amount of light, not to its quality.

The most important of all SENEBIER's discoveries was, however, that the presence of carbonic acid proved a deciding factor in the development of oxygen by green plants. His theory of this phenomenon was that the carbonic acid was dissociated and thus the oxygen liberated. This was proved by three important facts: a, In distilled water, leaves developed no oxygen (already pointed out by BONNET (1754)); b, the amount of oxygen rose and fell with the amount of carbonic acid in the water within certain limits; c, carbonate of lime gave off no oxygen in distilled water, but if some acid was added which liberated the carbonic acid in this water, green leaves, when introduced and exposed to sunlight, would develop oxygen.

These data constitute SENEBIER's chief contribution to the solution of the problem. He did not, however, stop at this point. He assured himself that the gas which was dissociated by the action of green leaves was the so-called "fixed gas" (carbonic acid), the amount of which, in the water, decreased considerably as the leaves acted upon it. The leaves separated the useless from the useful admixture, expelled the "pure air," i.e., the oxygen, and absorbed the phlogiston as an element in their development and growth. He further concluded that the metabolism of plants consisted in the association of carbonic acid, plant juices and light, this process being confined to green tissues, probably to their resinous elements (i.e., the chlorophyll). The juices of plants were replenished through the roots, which absorbed water, some solid matter, and carbonic acid (dissolved in the water).

The source of the exhaled oxygen was an important problem, and SENEBIER at once asked whether it might be attributed to atmospheric carbonic acid. His reasoning on this point is very interesting: "Carbonic acid is, and always must be, present in the lower strata of the atmosphere . . .

the problem is whether it may enter into the leaves. I confess that I do not believe carbonic acid can be carried into the leaves as a gas . . . but that it enters after having been dissolved in the water, like in charged waters."

His next conclusion was that the carbonic acid, thus present in the plant tissues, was decomposed by sunlight; the phlogiston (carbon) united with the "resinous substances" (chlorophyll), which have a great affinity for carbon.

His most important conclusion, however, was: "If the amount of oxygen given off by the leaves, is proportionate to the amount of carbonic acid in the water, and if the leaves in the water have absorbed only the carbonic acid contained therein, then the gas which is produced must be the result of a dissociation of the carbonic acid."

Scarcely less elucidating was his next conclusion, that the service of the carbonic acid lies in assisting in the formation of the acid substances contained in the plant.

Thus, in 1788, SENEBIER recognized—naturally on the basis of LAVOISIER'S analysis of carbonic acid—that "phlogiston" was identical with carbon; that carbonic acid is dissociated within the plant tissues; and finally, that the plants serve as regulators of the atmospheric content of carbonic acid.

In 1792 this subject was resumed in a paper in the *Journal de Physique*.

In this paper he confirmed his former findings with the following addition: "In regard to the formation of the amount of hydrogen necessary for the production of oils and vegetable acids, this is doubtless due to the dissociation of the water, but experience has not yet taught me how it takes place in the plants." This shows that SENEBIER at this time considered water not only a medium of solution from which plants might extract certain gases dissolved therein, but as a nutritive element, subject, in itself, of an advantageous dissociation.

Substantially the same conclusions were deposited in the *Physiologie végétale*, 1800.

As is well known, INGEN-HOUZS never recognized the fact that the secretion of oxygen depends on the presence of CO_2 in the plant. The controversy between these two investigators, at times very bitter, was continued for many years. The details, embodied in more or less tart papers, cannot now interest us deeply. To INGEN-HOUZS belongs the credit of explaining PRIESTLEY'S discovery that living green plants "improve" the atmosphere in such a way that it will increase combustion and sustain the life of animals. This was abundantly (and independently) confirmed by SENEBIER. Neither had perfected his experiments far enough to prove conclusively that CO_2 is developed by plants in the absence of light. The share of each of these two men in the discovery of almost identical facts has been subject of much discussion and even controversy; the fact remains that INGEN-

Housz admitted the development of oxygen by chlorophyll-bearing plants without the presence of CO_2 . But neither *proved* the assimilation of CO_2 from the atmosphere, even though INGEN-HOUSZ observed and recognized it. Either, however, *carried Priestley's and Bonnet's observations into the field of exact experimentation*, and but few fundamental discoveries in plant physiology have been as carefully and persistently documented.

SENEBIER's studies of the cause of etiolation were quite exhaustive, but barren of valid result. More successful were his studies on the sleep-movements (afterwards continued by his friend A. DE CANDOLLE), by which it was proved that a degree of turgor remains even amidst the periodic movements.—J. CHRISTIAN BAY, *John Crerar Library, Chicago, Illinois.*

GRAFTING EXPERIMENTS WITH COTTON¹

(WITH ONE FIGURE)

A successful method of reproducing cotton asexually offers interesting possibilities in retaining parental genotypes of this crop indefinitely. In previous papers the author has described his attempts to propagate cotton by stem cutting.^{2,3} More recently some preliminary experiments in grafting cotton have been completed and it is desired to present the results of this work in the present paper. While no attempts have been made by the author to try budding in connection with the propagation of cotton, McNAMARA and HOOTON⁴ of the U. S. Cotton Breeding Station at Greenville, Texas, have succeeded in propagating cotton by budding. Grafting as a method of reproducing cotton asexually has received the attention of the writer only after poor results were secured with cuttings. Although numerous attempts have been made to root cotton stem cuttings, less than 10 per cent. of the cuttings resulted in new plants. Since little or no difficulty was experienced in securing a high percentage of callusing in stem cuttings, it was expected that grafting might be very successful.

The saddle graft method was used in these trials, fig. 1.⁵ The main stem of the cotton plant to be used as the stock was trimmed to a slender wedge immediately above the lower node. Any leaves or branches below this node were removed. A scion of medium mature wood and of similar diameter to the stock was selected and cut to retain three nodes. In preparing the scion a cross-sectional cut was made immediately below a basal bud. The lower end of the scion was then split a short distance and fitted over the wedge of the stock so that the cambium layers of the scion and stock were matched

¹ Contribution from the Division of Agronomy, Texas Agricultural Experiment Station. Approved by the Director as Technical Contribution no. 130.

² REA, H. E. Asexual reproduction of cotton. *Jour. Hered.* 19: 356-357. 1928.

³ REA, H. E. Callusing of cotton stem cuttings. *Plant Physiol.* 5: 575-585. 1930.

⁴ McNAMARA, H. C., and HOOTON, D. R. Unpublished data.

⁵ BAILEY, L. H. *The nursery book.* Macmillan Co. 1912.



FIG. 1. Saddle grafts of cotton showing (A) enlarged view of well developed union, (B) growth of scion 15 days, and (C) 71 days after grafting.

at least on one side. After placing the scion the complete graft was wrapped with paraffined cotton string and sealed with warm paraffin. Several weeks later, after the scion had put out a leaf bud and the physiological union appeared to have taken place, the string and paraffin were removed and all leaf buds on the stock prevented from developing.

Using the saddle graft method 188 trials were made during the summer of 1930. Of this number of grafts 87, or 46.2 per cent., were successful and resulted in the production of vegetative growth and fruiting of the scion. Scions were placed at seven different dates as is shown in table I. The trials made on a specific date were comparable; however, the scions placed on the several dates were under varied conditions.

On May 14, June 25, August 9 and August 21, a total of 38, 32, 18 and 13 grafts, respectively, were made under similar conditions. The scion and stock used on these dates were all of the Lone Star variety of cotton but in each case the scion was of a different plant from the stock. The grafting was accomplished early in the morning while the temperature was moderate and the work was usually completed prior to 9 A. M. Each scion and stock was prepared and the graft completed before disturbing the next plants. Using this procedure 63.2, 75.0, 72.2 and 84.6 per cent. of the grafts attempted on May 14, June 25, August 9, and August 21, respectively, were successful. In all, 101 plants were grafted in this series and 72 scions grew, making an average of 71.3 per cent.

This was by far the most successful lot of grafts made in these preliminary trials. The plants used as stocks on May 14 were growing in 8 inch flower pots on the greenhouse bench. On September 4 at an age of 113 days the scions used on these plants were only 10 inches long but had blossoms and half mature green bolls on them. The plants used on June 25 were in 16 inch wooden tubs and had made considerably greater vegetative growth

TABLE I
SUMMARY OF PRELIMINARY GRAFTING TRIAL WITH COTTON

DATE OF TRIAL 1930	CONDITION OF TRIAL	PLANTS SUCCESS- FULLY GRAFTED	PLANTS USED	SUCCESSFUL GRAFTS
May 14	Scion and stock of Lone Star, cut immediately before use. Work accomplished prior to 9 A. M.	24	38	63.2
June 25	“	24	32	75.0
August 9	“	13	18	72.2
August 21	“	11	13	84.6
Sub total	“	72	101	71.3
July 17	Scion of Willet Red Leaf and stock of Lone Star. Scion cut 48 hours prior to use and re-cut just prior to placement. Work accom- plished prior to 9 A. M.	None	19	0.0
August 13	Scion and stock of Lone Star. Scion cut 1 hour prior to use. Work accomplished prior to 9 A. M.	7	29	24.1
August 20	Scion and stock of Lone Star used immediately after cut- ting. Work accomplished from 11 A. M. to 3 P. M.	8	39	20.5
Grand total	For all trials	87	188	46.2

than the early lot. The stems on some of these plants were 30 inches long. The development of the other lots of grafts varied all the way from the opening of a single leaf bud to three or four leaves. The information regarding the growth of the scion is offered only to show that the scion made normal development corresponding to the environment of the stock.

In the case of 19 scions of Willet Red Leaf cotton, placed on a like number as Lone Star stock on July 17, all died. These scions had been transported a considerable distance before being used and were cut from the parent plants 48 hours prior to placement on the stock. Although the scion wood was kept in loosely closed moist glass jars and a fresh cut was made on each scion as it was used, it is supposed that the delay injured the scions. On August 13 there was a delay of one hour between the time the scion-stick was cut from the parent stalk and placed on the stock. This treatment resulted in only 7 out of 29 of the scions growing, or 24.1 per cent. Again

on August 20 a total of 39 scions were placed under conditions thought to be injurious. This time the grafting was done during the heat of the day from 11 A. M. to 3 P. M. Only 8, or 20.5 per cent., of these scions grew.—*H. E. REA, Substation no. 5, Texas Agricultural Experiment Station, Temple, Texas.*

TEMPERATURE AS A POSSIBLE FACTOR IN REGENERATION

In view of some recent publications on the subject of regeneration the writer believes that he is justified in mentioning some observations made in 1926 while at the University of New Hampshire.

The Botany Department had two greenhouses in operation at that time, one maintained at a nearly constant temperature of 18° C. and the other at 15° C. For a time the temperature of the warm house was run above 25° C. for as much as four hours a day, but the cold house was not allowed to run above 20° C. During the course of other work the writer decided to see if temperature could have any effect on the regeneration of buds from tomato hypocotyls where the tops had been removed. Tomato was chosen because other members of the family had given striking examples of such regeneration after such operations had been performed while tomato itself refused absolutely to give any such results under normal conditions.* Accordingly pans of seedlings of tomato were prepared and started under normal conditions. When these had reached a good size, the seedlings were carefully clipped off well below the cotyledons, care being taken to see that no primordial buds such as are generally found in the axils of cotyledons remained. Checks were also kept of uninjured seedlings. Certain seedlings were then placed in the "hot" house, and an equal number in the "cold" house, each with their checks.

In a short time the writer was interested to note that practically every one of those in the "hot" house regenerated their tops by means of shoots arising from the top of the hypocotyl and thriftilly produced a new set of tops. On the other hand, not one seedling of the "cold" house regenerated a new shoot or even produced a single bud. The best that they seemed able to do was to turn green and to remain alive for a time. All of these finally died. The experiment was repeated again, with the same results.

From these results the writer is inclined to believe that temperature plays a part in the regeneration of injured parts, at least in the tomato.—*FRED R. CLARK, Southeastern State Teachers' College, Durant, Oklahoma.*

* CLARK, FRED R. Bud formation on plant hypocotyls. *Ann. Rept. Michigan Acad. Sci.* 20: 146. 1918.

NOTES

Cleveland Meeting.—The seventh annual meeting of the American Society of Plant Physiologists at Cleveland was very largely attended, and was most successful in every way. The audience room in the Law Building of Western Reserve University was crowded most of the time during the sessions, and the papers were in the main interesting and valuable. The business sessions showed that the Society is growing substantially, and that the financial foundations are satisfactory. A spirit of optimism pervaded all of the sessions; and the banquet, at which nearly a hundred of the members were gathered, was an occasion long to be remembered. It was made the occasion of the second award of the STEPHEN HALES Prize, and the sixth award of the CHARLES REID BARNES Life Membership. With this meeting in the background, plans will no doubt go forward at once for a larger and better meeting, if possible, at New Orleans in December, 1931.

Pasadena Meeting.—The American Association for the Advancement of Science will hold its summer meeting at the California Institute of Technology, at Pasadena, in June. Tentative plans are being drawn for the participation in this meeting by the western members of the American Society of Plant Physiologists. Announcement of more details may be expected in April.

Life Membership Award.—The committee in charge of the sixth award of the CHARLES REID BARNES Life Membership in the American Society of Plant Physiologists bestowed that honor upon Dr. RODNEY HOWARD TRUE, Professor of Botany in the University of Pennsylvania, a life-long investigator of important phases of plant physiology. Dr. TRUE was born at Greenfield, Wisconsin, in 1866. His earlier training was at the University of Wisconsin, but his doctorate work was completed at Leipzig, with PFEFFER, in 1895. While at Wisconsin, he had been a student with Dr. BARNES, a circumstance which was made known by Dr. TRUE in his gracious reply to the announcement of the award. After returning from Germany, Dr. TRUE was for some years a member of the Department of Pharmacognosy at Wisconsin, and for a brief time, lecturer at Harvard University. Beginning about 1901, he was for nearly 20 years in charge of the physiological investigations of the Bureau of Plant Industry. He built up a strong organization for investigation of fundamental problems of plant life. His work on nutrition of plants, with special reference to calcium in relation to the intake of the other mineral elements, was of great signifi-

cance. In 1920 he resigned this work to become Professor of Botany and Director of the Botanic Garden, University of Pennsylvania. During a number of years he has been secretary of the Committee of One Hundred on Scientific Research, of the American Association for the Advancement of Science.

A pleasing feature of the announcement was the fact that the presentation of the award was made by Dr. R. B. HARVEY, of the University of Minnesota, who had been at one time associated with Dr. TRUE's work in the Bureau of Plant Industry. Dr. TRUE's response was in delightful vein, recalling his earlier connection with Dr. BARNES at Wisconsin.

Stephen Hales Prize Award.—The second award of the STEPHEN HALES Prize was made to Dr. WIGHTMAN WELLS GARNER, chemist and plant physiologist of the U. S. Department of Agriculture, who has been for many years in charge of the tobacco and plant nutrition investigations of the Bureau of Plant Industry. Dr. GARNER was born at Timmonsville, South Carolina, in 1875. A graduate of the University of South Carolina, he received his Ph.D. from Johns Hopkins University in 1900. He began his work in the Bureau of Chemistry in 1904, and became chief of the tobacco and plant nutrition work in 1909. He is known internationally for his work on photoperiodism and related phenomena.

Changes of Address.—All members of the Society should register changes of address promptly with the Secretary of the Society. PLANT PHYSIOLOGY is an expensive journal, and lost numbers break volumes, which in broken sets are worth only a fraction of their real value to the treasury. To prevent failure to deliver, notify the Secretary, and leave a forwarding address with your local postmaster. Even so, there is delay in delivery until the member sends the necessary postage for forwarding. Much trouble will be saved for everybody concerned by sending the Secretary both the old and the new address, and requesting an immediate change in your addressograph stencil.

Portraits.—Beginning with this issue, PLANT PHYSIOLOGY presents a series of portraits of famous contemporary plant physiologists. It is a great pleasure to begin this series with the portrait of Dr. LUDWIG JOST, of the Botanical Institute of Heidelberg University. In April, we hope to have a reproduction of an excellent crayon of Dr. F. F. BLACKMAN, of Cambridge University. If it can be arranged, the July and October numbers will carry portraits of Dr. F. A. WENT, of Utrecht, and Dr. N. A. MAXIMOW, of Leningrad. These are in addition to the biographical portraits which appear from time to time. In this issue we have a rare por-

trait of SENEBIER, furnished by Dr. BAY, of the John Crerar Library. Portraits of all plant physiologists appearing in PLANT PHYSIOLOGY can be obtained at 12 cents each. In complete sets, or in sets of 9 or more, the first 9 will be furnished for \$1.00, postpaid. Orders should be sent to the editor, as these portraits are held at the editorial office, in Chicago.

Endowment Funds.—The American Society of Plant Physiologists has three funds to which gifts can be made at any time by those who desire to share in the permanent support of the Society's program. The program is flexible enough to permit constant adaptation of the use of funds to changing needs, so that no one need fear that funds might some day be useless. First, the STEPHEN HALES fund, which now amounts to \$1100, for award of occasional prizes for outstanding contributions to the knowledge of plant physiology. Several hundred dollars should be added to this fund to guarantee income sufficient for awards at least biennially. Second, the CHARLES REID BARNES Life Membership fund, which has been allotted \$1400 by action of the Executive Committee. With the provision made last year, this fund is ultimately guaranteed completion. At the same time, the way is left open for anyone who was formerly associated with BARNES, or who would consider it a privilege to share in honoring BARNES in this way, to make gifts to the fund. As life memberships are vacated, enough of these are to be added to the principal fund to bring it up to the amount required for an annual award. Third, the general endowment fund, established to care for any extraordinary service, such as publishing expensive papers, monographs, colored plates when necessary, etc. This fund has been started during the year, and contains \$110. Friends of the science of plant physiology are invited to share in these projects whenever they feel like doing so. Financial strength will enable the Society to proceed with complete confidence in the future. All gifts should be sent to the Secretary-Treasurer, Dr. WRIGHT A. GARDNER, Alabama Polytechnic Institute, Auburn, Alabama.

Back Number Wanted.—Volume I, Number 1, January, 1926, of PLANT PHYSIOLOGY is wanted by the New York State Agricultural Experiment Station, Geneva, New York. The library of the New York Station is very anxious to purchase Volume I, Number 1, of this journal to complete its file. Members and subscribers are urged to examine their files for a spare copy of this number, and communicate with Dr. JAMES G. HORSFALL, who will arrange for its purchase. The Station will appreciate greatly the cooperation in this matter of members and subscribers.

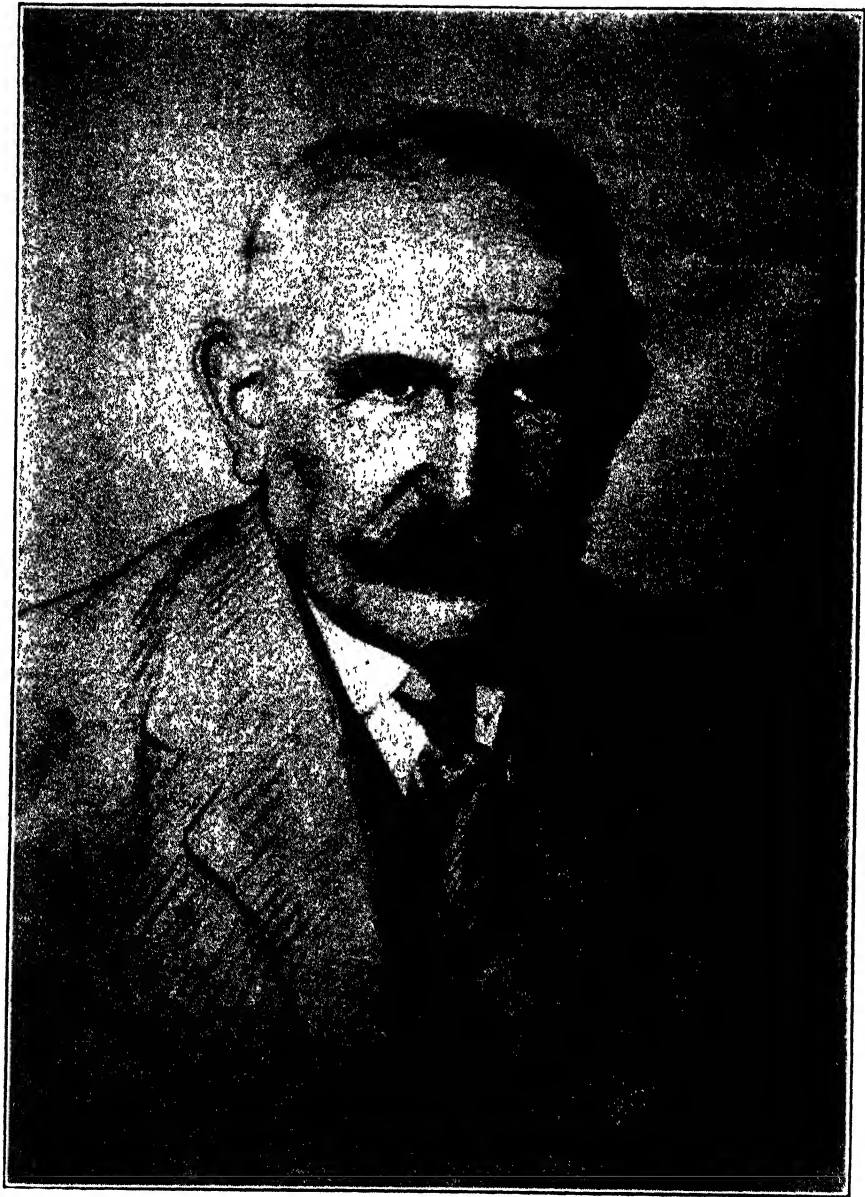
International Critical Tables.—The final volume of the first edition of the International Critical Tables was sent out by McGraw-Hill Book Co. late in 1930. This volume (VII) contains data on refractivity of gases and vapors, elements, selected solids and liquids, doubly refractive solids, organic liquids, mixtures, and electric and magnetic birefringence; kinetics of chemical processes, of biochemical reactions, and of photochemical processes. The latter section presents data on filters for Hg-vapor lamps, reaction velocities of photochemical changes, temperature coefficients, displacement of equilibria, phototropy, and quantum sensitivity. Succeeding sections consider absorption spectra of dyes, viscosity of pure liquids, free energy of chemical substances (thermodynamics), solubility of slightly soluble salts in aqueous solutions of electrolytes, optical rotatory power of solid crystals, optical rotatory power of liquids and solutions, and commercial explosives. At the close is a list of journals and books with key numbers for references. A separate index in English, French, German, and Italian covering all seven volumes, is furnished with volume VII. The publishers also announce a continuation with Annual Tables of Constants and Numerical Data, vol. VIII for 1927–28, IX for 1929, and X for 1930. Subscribers for the earlier volumes are allowed discounts for the later ones if ordered promptly.

A Textbook of Plant Physiology.—A welcome addition to the few available textbooks of plant physiology is the translation of N. A. MAXIMOW's textbook, edited by Dr. A. E. MURNEEK and Dr. R. B. HARVEY. This work is published by the McGraw-Hill Book Co., at \$4.00 per copy. The book is divided into four sections: Absorption of matter and energy; Water relations of the plant; Utilization of reserve products and liberation of energy; and Growth, movement and reproduction. There are only twelve chapters in all. The careful editing has removed the evidences of translation, so that it will give the impression of an English text. It is written in clear style, is well illustrated, and should prove useful as a text. Those who prefer to begin with intake of water and salts can easily transpose the order of chapters for classes. The water relations section is valuable as a summary of MAXIMOW's theoretical conceptions and research in this field.

Enzymes.—Longmans, Green and Co. have issued a new book in their "Monographs on Biochemistry" series. It is entitled *Enzymes*, and is by Dr. J. B. S. HALDANE, of the Department of Biochemistry, Cambridge University. As it is based on a lecture course, it lays no claim to completeness, but has to do more with the biochemical nature of enzymes and their

actions. After an introduction, HALDANE takes up the influence of enzyme concentration and hydrogen ion concentration upon the rate of reactions. The succeeding chapters are as follows: The union of the enzyme with its substrate and related compounds; the influence of temperature and radiation on enzyme action; the course of enzyme reactions, and its mathematical theory; specificity; coenzymes, activators, kinases, and complements; the poisoning of enzymes; the purification and chemical nature of enzymes; theories of enzyme action, and classification of enzymes. Over 500 citations precede the index. The book is an interesting summary, and is more valuable than if it were crowded with a multiplicity of details from attempts to be more complete. It is based upon and extends the field cleared by BAYLISS in his earlier monograph, but without attempting to revise the earlier work. It is a valuable contribution in its field. The price of the work is \$5.50, and orders should be sent to the publishers, New York.

Elements of Plant Science.—Under this title Dr. CHARLES J. CHAMBERLAIN of the University of Chicago presents an elementary textbook of Botany for beginners. The work is divided into two parts, the first part dealing with the structures and functions of seed plants, and the second with development of plants from the lowest to the highest. It is illustrated with 321 figures, and is a fresh presentation by one who has devoted his lifetime to a study of plants. A series of laboratory directions and exercises accompanies each section. Teachers of beginning botany will find much that is helpful in this book, and students will find it an attractive and stimulative introduction to plant life. It is published by McGraw-Hill Book Co., and the price, \$1.90, is attractively low. Most students and investigators in the botanical fields will find it an interesting review of the fundamentals of plant life, and the organization of the plant kingdom.



FREDERICK FROST BLACKMAN
1866

READER IN BOTANY, THE UNIVERSITY OF CAMBRIDGE

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AN EXPERIMENTAL STUDY OF THE GERMINATION OF WHEAT SEED UNDER WATER, AS RELATED TO TEMPERATURE AND AERATION*

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(WITH FIVE FIGURES)

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Introduction

THE PROBLEM

The environmental complex operates conjointly with the internal characteristics of an organism to control physiological activity. The number of kinds of environmental influences that may act on a living thing is of course very large; it may indeed be regarded as infinite, for it includes, among other things, not only all the kinds of molecules and ions that may possibly act to alter the course of physiological activity in any way, but also as many partial ranges of radiation frequency as one chooses to consider. Each kind of influence may act with any number of degrees of intensity and intensity may fluctuate in any number of ways in the period of the life of the organism or throughout any developmental phase. Also, the time intervals considered in studying developmental changes may have any number of different lengths within the life period. Finally, the complex of all the environmental conditions that influence an organism for any time period may have any number of physiological values or capacities because of differences in the manner in which the component influences are combined or are concomitant.

Starting with a given set of internal conditions, as those of a seed placed in culture for germination, the course of development, or whether development shall occur at all, must be considered as determined by the environmental complex and the physiological capacity of the seed operating together. To appreciate the behavior of a germinating seed it is consequently necessary to take into account not only the whole of the influential complex acting from outside the seed but also the capacity of the seed to respond to or to withstand environmental influences. For a given set of internal conditions (kind of seed, health, vigor, etc.) different response patterns are brought into being for different combinations of effective environmental conditions and their various intensities; provided, of course, that the environmental differences considered are sufficiently great and prevail for sufficiently long periods of time to show noticeable corresponding differences in the behavior of the awakening organism.

To approach an experimental analysis of the conditional control of any kind of organism the problem needs to be attacked by the employment of relatively simple systems—the simpler the more promising—and this statement applies to the kind of organisms to be studied as well as to the environmental complexes that are to be employed. It is also desirable, in the beginning, to study physiological processes that lend themselves most readily to measurement and to comparison with respect to process rates and their end results. The general nature of the relations between organisms and their environments has recently been discussed from this view-

point by LIVINGSTON (12). Somewhat the same viewpoint was in part taken by F. F. BLACKMAN (3), in his classic discussion of environmental optima and limiting conditions.

Most students of the environmental relations of organisms have dealt with systems far too complicated for first steps in such analyses. The problems have in many instances involved too many experimental variables and many of these have failed to lend themselves to adequate numerical description. It has also occurred in many cases that the background variables (those not intended to differ in intensity from test to test) were not described sufficiently well to permit satisfactory approach to duplication if an experiment were to be repeated. These conditions of the experimental background might frequently differ from experiment to experiment and might fluctuate during the experiment periods in unknown and consequently unreproducible ways. Also, the several different intensities of the experimental variables themselves (the ones that are intended to differ in intensity from test to test) have been chosen in many instances so as not to represent adequately the whole range of intensities dealt with. To illustrate: only a few different maintained temperatures might be employed in a study of the temperature relations of a physiological process, when many additional temperatures would be needed to supply the points necessary for the construction of the ordinary temperature curve; the non-temperature conditions of the background might be inadequately described and the length of experiment period might not be chosen so as to bring out the time relations in a consistent manner.

The main purpose of the study to be reported in this paper was to attack a relatively simple set of physiological relations by use of a comparatively simple set of variables. The plant material chosen was a stock of wheat seed, whose germination capacity was to be studied in relation to various sets of environmental conditions. Resting seeds are relatively simple organisms in many respects, at least they are much simpler than seedlings or plants in later developmental phases. Wheat seed is known to retain its viability or capacity for germination without great alteration for rather long periods of time and the variability of a lot of pure-line wheat seed only a year or two old has been found to be not nearly so troublesome to the experimenter as is the variability of lots of some other kinds of seed. Although seed germination is a complex process, yet it is much simpler and more easily measured than subsequent phases of plant development and an end point for the germination phase may be chosen so as to be easily identified. Of course it is clear that the farther the developmental process is allowed to go the more complicated must be the environmental relations, and consequently this study dealt with the process of germination only, from the beginning of soaking to the bursting of the seedcoat.

As to environmental conditions, it is of course necessary that all essential ones for the process studied shall be represented. For the germination of wheat seed these are comparatively few. Light is not essential and the very complicated question of light relations is set aside at once by limiting the problem to germination as it occurs in darkness. Water relations are readily dealt with by having the seeds always under dilute nutrient solution of a 3-salt type, which is very simple in comparison with many other media suitable for seed germination. While wheat seed does not germinate well in distilled water, different proportions of the nutrient salts in a dilute SHIVE solution appear to be without marked influence on germination and the growth of very young seedlings, as was pointed out by GERICKE (6). Maintained temperatures are rather easily arranged in experimental studies that do not involve light. Letting the seeds germinate always under weak aqueous solution not only simplifies the question of water relations, as has been said, but it also simplifies the question of the influence of aeration. Natural aeration in a stagnant solution culture is not very vigorous and different degrees of aeration (including stirring of the solution) may readily be arranged by means of a bubbling air stream.

The duration factor, which is always troublesome in experimentation on environmental influence, was simplified by having the longest experiment period only 24 hours, which markedly limited the possible influence of unknown changes in the medium as well as the extent of fundamental changes in the organisms during an experiment. This question of the influence of alteration in the influential conditions is of paramount importance and it would surely require serious attention if the seedlings were allowed to advance beyond the bursting of the seedcoat or if the experiment periods were much longer than the longest one employed in this study. By the use of three experiment periods shorter than 24 hours data were secured which may be useful in case later studies make the 24-hour period appear too long for the other features of the experimentation, especially with reference to unknown progressive changes in the nutrient medium—such as absorption of salts or ions and leaching or excretion of material from the seeds.

Seed germination was chosen as the subject for this study primarily because it constitutes a readily measured physiological process reduced nearly to its lowest terms of complexity. But there are other good reasons for further experimental study of the viability of a lot of seed. Practical methods for seed testing must be based on the principles to be brought out by such experimentation as this and the more we know of germination in general the more rapidly can seed-testing procedure be improved.

Many experimental studies on the relations of more advanced developmental phases of plants involve the use of plant material secured from

seeds—indeed, for higher plants experimental material must be derived from seeds unless cuttings or other means of vegetative propagation are employed. And one of the main desiderata for any kind of experimentation with plants is that the different individuals of the stock of plants used should be as nearly alike as possible. Whenever seedlings or subsequent stages of development are to be employed it is necessary that serious attention be given to the conditions that prevail during seed germination and it appears that one of the most promising ways to procure a lot of reasonably similar plants is to carry out selections based on germination and the very early phases of seedling growth. Thus, the results of a study such as the one here reported may be valuable in a fundamental and very practical way, as furnishing bases for many kinds of experimental study of more advanced phases.

Because seed germination constitutes a conspicuous example of the awakening of plant organisms from a dormant condition, after all physiological processes have been at very low ebb for a long time, this kind of growth is itself of special importance in general physiology.

The present study was carried out at the Laboratory of Plant Physiology of the Johns Hopkins University, from the fall of 1929 to the spring of 1930. In the planning of the experimentation and in the assembling and presentation of the results the writer has been guided and helped by Professor BURTON E. LIVINGSTON, director of that Laboratory, to whose interest this paper is largely due.

EARLIER STUDIES ON SEED GERMINATION

Ever since the earliest days of botanical science seed germination has been studied, sometimes from the viewpoint of physiology but in most cases with regard to agricultural and horticultural practice, and the literature of this general subject is very extensive. Recent contributions on seed germination may be classified in two categories: (1) Studies undertaken chiefly with reference to seed testing, for comparing different lots of seed in regard to their various degrees of viability or germinative energy. To this group belongs the work of HARRINGTON (8) and WILSON (27). (2) Studies on seed dormancy and on special treatments by which the dormant period may be shortened. Here belongs the work of CROCKER (4), ATWOOD (1), HARRINGTON (9) and MORINAGA (19). Most of the more recent studies are more satisfactory than earlier ones, but the interaction of the organism and its environment deserves more thorough attention than has been given to it. Experimentation on seed germination has generally dealt with the behavior of the organisms in relation to rather limited ranges of artificially controlled or measured experimental conditions and little attention has been paid to the other variables, which are of course influential along with the experimental ones.

Several contributions to our knowledge of this subject should be mentioned here, attention being confined to the few papers that deal with germination from viewpoints more or less similar to that of the present study or that furnish information pertinent to the interpretation of the results to be reported in subsequent sections of this paper. In a study of optimal temperatures for seed germination, employing seeds of 18 different plant forms, a series of maintained temperatures and a number of different substrata, HARRINGTON (8) ascertained the temperature and the length of time best suited for testing the viability of the kinds of seed studied. The results show how different kinds of seed differed in promptness of germination and how different temperatures differed in their influence on different kinds of seed. These results are of course referred to the ranges of influential conditions that were included in HARRINGTON'S experiments, but it is impossible to gain a clear idea of his prevailing non-temperature conditions because these were not controlled in detail and their description could not be made as specific as would be necessary if these tests were to be repeated.

WILSON (27) studied the germination of wheat seed on moist plaster-of-Paris plates at different maintained temperatures, continuing each test till no further increase in the number of germinated seeds was observed. The number of days required for the earliest and for the latest germination was recorded in each instance. Some of his resulting data are shown below, for five different maintained temperatures.

	10°	15°	20°	25°	30°
Average germination percentage	93.5	96	94	90	78
Average no. of days required for earliest germination	5	4	2.5	2	1
Average no. of days required for latest germination	11	10	6.5	4	4

From these data WILSON concluded that the optimal temperature for germination was 15°, because that temperature gave the highest average germination percentage at the end of the experiment. For 15° the final percentage was 96 and the period was 10 days, but nearly as high percentage values were obtained at temperatures of 10° (93.5 per cent. in 11 days), 20° (94 per cent. in 6.5 days) and the final percentage for 25° was of the same order of magnitude (90 per cent. in 4 days). In

studying these results it is surely permissible to consider 93.5, 96 and 94 as alike, and it seems clear that, when we consider the duration factor, 20° must be regarded as much more nearly the general temperature optimum than 15°. At any rate, it is clear from the tabulation just presented that any temperature between 10° and 25° might be expected to give nearly complete germination in a lot of seed like that used by WILSON.

MORINAGA has recently reported a series of studies on germination (19, 20, 21) and those devoted to germination under water are of special interest in connection with the present report. In each of these water experiments the seeds were on the bottom of a 100-ml. Erlenmeyer flask filled with distilled water and kept in a greenhouse at a temperature fluctuating between 15° and 35°, the water being renewed every two weeks. A seed was considered as having germinated as soon as the hypocotyl or plumule showed notable enlargement. Out of 78 plant forms tested, the seeds of 43 germinated under water and 18 of these showed no decided differences between germination in water and germination on wet filter-paper. For the remaining 25 germination was more complete or more rapid on wet paper. MORINAGA reported that his wheat seed was unable to germinate under water, thus agreeing with similar results secured by KRAUS, who found, according to MORINAGA, that his wheat seed did not develop under water farther than to the stage at which the coleoptile protruded. In some of MORINAGA's experiments wheat seeds, as well as seeds of some other kinds that failed to germinate under water, were able to do so when nearly pure oxygen replaced the air above the water. This apparent influence of aeration in connection with seed germination under water is surely important.

In one of MORINAGA's studies (19) 10 samples of 10 seeds each of white clover were tested under water and a similar series of samples was tested on moist filter paper in Petri dishes, at maintained temperatures of 5°, 10°, 15°, 22°, 27°, 32° and 38°. The germination percentages were recorded after 2, 6, and 10 days. The results show a number of remarkable things, some of which were pointed out by MORINAGA. When germinating under water the optimal temperature for this white-clover seed lay between 15° and 27° for all incubation periods studied, but the corresponding filter-paper tests gave a temperature optimum about 22° for a 2-day period and an optimal range of 10°–22° for the longer periods. There was thus a broadening of the optimal temperature range with longer time of incubation when the seeds were on filter paper, a phenomenon apparently related to the shifting of the optimal temperature shown by WILSON's data as well as by those of HAASIS, which will be referred to later on. Had MORINAGA chosen more and shorter time intervals he would probably have obtained a notable downward shifting of the optimal temperature for his filter-paper cultures as the incubation period became longer. The minimal temperature

for germination, for both water and filter-paper cultures was about 5° for the 2-day period and for longer periods it was considerably lower. The maximal temperature for germination was not shown for any period with the seeds under water, being always well above 38°, but it was apparently not much above that temperature when the seeds were on wet filter paper. Furthermore, the rate at which MORINAGA's lot of white-clover seed germinated in water is much more rapid than the corresponding rate for cultures on filter paper.

By studying the germination of seeds under water, MORINAGA certainly advanced a great step beyond earlier workers in this field. His moisture and aeration conditions were thus much more definitely specified than is possible with other methods of treatment. Since his incubation periods were rather long it might have been better to renew the water oftener than at 2-week intervals, but each time the water is renewed in such cultures the seeds are rather thoroughly aerated for a short time. A continuous flow of the liquid medium might be valuable in such experiments, if it could be arranged, and that might care for the maintenance of aeration conditions as well as of other solution characteristics.

MORINAGA suggested that the differences which he observed between germination in distilled water and on wet paper might be related to oxygen supply and the accumulation of toxic products of respiration. Such products might not accumulate so much in seeds surrounded by liquid medium as in seeds on wet paper; for the liquid medium acts to remove excreted material from the immediate surroundings. The so-called poison action of distilled water may perhaps deserve consideration in this connection. It has been studied by many writers, among them LOCKE (16), RINGER (23), OSTERHOUT (22), LIVINGSTON, *et al.* (14), TRUE (26), MERRILL (18) and GERICKE (6). There seems to be no doubt that a weak nutrient solution is, in general, more satisfactory as culture medium than distilled water is. Distilled water is never quite pure and it may influence an organism in contact with it by supplying the absorbing cells with influential ions or molecules in extreme dilution, as Cu or Zn or organic substances that distill with steam. This possibility is difficult to avoid. Distilled water also differs from a nutrient solution in that it furnishes no considerable diffusion tension of the essential inorganic ions and therefore generally permits more loss of substances from the organism than would occur if a good nutrient solution were employed. Finally, ions and molecules leached from the absorbing cells may accumulate in the medium and, with or without modification, these may subsequently exert considerable influences upon the organism. Leaching appears to occur more rapidly when seeds are under distilled water than when they are under a fairly well balanced nutrient solution.

HAASIS (7) studied the germination of seeds of several species of coniferous tree with special reference to the influence of incubation temperature and length of the incubation period. He emphasized the importance of defining or specifying all the influential background conditions of an experiment, although these do not enter directly into consideration as experimental variables. He also emphasized in a new way the importance of the time factor in physiological processes. His results with pitch pine seed are specially interesting in the present connection. Employing an extensive series of maintained temperatures ranging from 16° to 57° and incubation periods ranging in length from 6 or 7 hours to 14 days, he observed that the optimal temperature for the germination of his pitch pine seed shifted from about 47° (for a 1-day period) to about 23° (for a 10-day period) apparently remaining unchanged for more prolonged incubation. With incubation periods of intermediate length there was evidence of two very different optimal temperatures, one at about 31° and the other at about 43°. This important observation would have escaped attention had the incubation periods of about 2 or 3 days been omitted from the plan of HAASIS's study. The same writer also emphasized for the first time the possibility of using a series of different germination treatments for the purpose of defining and sorting out several physiologically different classes of seeds from a given lot. HAASIS's technique did not allow very definite specification with regard to moisture and aeration conditions. All of his seeds were treated with hydroxy-mercuri-chloro-phenol ("semesan") to avoid mold growth. His experimentation was unusually well planned and his data for pitch pine seed are exceptionally complete and generally very consistent. Many interesting points are brought out in his discussion.

Methods and procedure

THE MAINTAINED TEMPERATURES

For maintaining the desired temperatures the series of seven chambers described by LIVINGSTON and FAWCETT (15) was employed. The apparatus stands in one of the greenhouse rooms of the Laboratory of Plant Physiology of the Johns Hopkins University. The chambers are vertical cylinders 28 cm. in diameter and 43 cm. high, each with a water jacket and a stirrer to keep the water in slow rotation. The whole series is well insulated from the outside air by means of packed ox hair and wood and each chamber has a removable lid made of wood and cork. Adjacent water jackets are separated from each other by vertical partitions of galvanized and asphalted sheet iron. Water does not move from one jacket to the next but heat is transferred through the partitions being supplied at one end of the series by means of a thermostatically controlled electric heater and removed at the other end by means of an electrically driven and auto-

matically controlled mechanical refrigeration machine. A thermal gradient is thus maintained between the two ends of the series and each of the seven chambers has its own maintained temperature, which fluctuated, in the present study, within a range of about plus or minus 1° C. Proceeding from the warm to the cool end of the row, each chamber is cooler than the next succeeding one. By suitable adjustment of the heating and refrigeration controls different series of maintained temperatures may be secured. For this study the temperatures employed were 12° , 19° , 24° , 30° , 35° , 40° and 45° C. The continuous, slow rotation of the jacket water around each chamber gives to all sides of the chamber practically the same temperature; the lower part of the air mass enclosed in a chamber was found to be generally a little cooler than the upper part but the difference between bottom and top was not over 2° . The culture flasks stood upright at the bottom of the chambers, all at the same level, accompanied by a thermometer or a thermograph, as space allowed.

GERMINATION FLASKS AND ACCESSORIES

Erlenmeyer flasks, of "Pyrex chemically resistant" glass 15 cm. high and with bases 9 cm. in diameter, were used in these germination tests, each flask containing 100 ml. of nutrient solution. The solution was 2.3 cm. deep and the diameter of its circular free surface was 8.5 cm., where it was in contact with the air. For the tests with flowing air each flask was closed by means of a 2-hole rubber stopper bearing an inlet and an outlet tube (inner diameter, about 5 mm.), the inlet extending downward nearly to the flask bottom while the outlet terminated just below the stopper. Three centimeters above the stopper each tube was bent at a right angle, with the horizontal arm 3 cm. long. In operation, each inlet tube was joined to the air-supply tube from its own wash bottle (in the same chamber with the germination flask) and all outlet tubes were joined to a line of tubing leading to an aspirator. All tubing was of glass, with short couplings of rubber tubing. In most of the experiments air from the greenhouse was continuously bubbled through the nutrient solution in each germination flask. At the end of each test the germination flasks were washed with cleaning fluid, thoroughly rinsed with distilled water and allowed to dry by draining before being used again.

AERATION OF CULTURES

For each germination flask with air flow there were two wash bottles of water, so connected that the air stream entering the flask had bubbled through them in series. The first of these (outside of the chamber) was a 200-ml. bottle provided with rubber stopper and two tubes, for inlet and outlet. The inlet tube opened into the greenhouse and the outlet led to the second wash bottle (inside the chamber). The latter was a 1-quart

"Mason" jar, with rubber stopper bearing inlet tube and outlet tube, the inlet leading from the first wash bottle while the outlet led to the flask. The first of these bottles was half filled with water and served primarily as a telltale, used in ascertaining from time to time the rate of air flow in terms of the rate of bubbling; it also served to increase the humidity of the air stream while the latter was still at greenhouse temperature. The second wash bottle (within the chamber) was about half full of water, at the maintained temperature of its chamber. This bottle served to bring the air stream to the desired temperature before it reached the germination flask. The air entering the flask was found always to have the temperature of the chamber in which the flask stood, even with the most rapid rate of air flow used and with the lowest and highest temperatures employed. In passing through the second wash bottle the flowing air was automatically brought to approximate vapor-pressure equilibrium with the dilute nutrient solution in the flask, so that evaporation or condensation in the flask was at a minimum.

The tube leading from each flask was provided with a special orifice, just outside the temperature chamber, for the controlled application of suction from the suction tank—on the principle that rate of gas flow is determined by pressure gradient and orifice resistance. This device was similar to the one used by HUTCHINS (10) for a like purpose, consisting of a plug of cotton wool and pulverized kaolin suitably packed in a slightly conical tube. Each plug was adjusted, by the proper degree of packing, to give the desired rate of flow with the suction employed. To prevent excessive condensation of water in the plug, which might increase its resistance to air flow, HUTCHINS kept the plug temperature always somewhat higher than that of the entering gas, but the same end was attained in the present experiments by means of a drying tube with calcium chloride, inserted between flask and plug inside the chamber. Five different orifice settings were used, with air-flow rates of about 1, 3, 6, 15 and 30 liters per day, or 40, 125, 250, 625 and 1250 ml. per hour. The corresponding numbers of bubbles passing through the telltales were 4, 12, 24, 60 and 120 per minute. The orifices were nearly alike for all flasks in the same experiment and the rates of bubbling in the flasks were similar to those shown by their respective telltales. The discharge tubes all led out of the temperature chambers to the suction tank, a 5-gallon bottle with rubber stopper, inlet tube and outlet tube, the latter leading to a "Cenco-Harrington" filter pump attached to the greenhouse water supply.

The nutrient solution used was of very low vapor pressure, its osmotic value being about 1 atmosphere for 20°, and neither condensation nor evaporation of water was observed to have occurred in any of the flasks, even at the end of the longest experiment period and with the most rapid

rate of air flow and the highest and lowest temperatures employed. This indicates that the second wash bottle in the air line leading to each flask operated effectively as both temperature and humidity control for the air stream. The surface level of the solution in the flasks was consequently always about the same (about 2.3 cm. above the flask bottom) and the submerged seeds were always at practically the same depth. The volume of solution around and above the seeds altered slightly, of course, through absorption and swelling, but water absorption and swelling by such seeds as wheat are almost equal in respect to volume. It is interesting to note that a hundred seeds had absorbed about 2.5 ml. of water when swelling was complete and that the volume of a swollen seed was about twice as great as its original volume before soaking. The total volume of seeds and water decreased by about 0.4 ml. in the first 24 hrs. of soaking. There were apparently some very small gas-filled spaces in the dry seeds, which became filled with water as soaking and swelling proceeded.

The air stream continuously supplied oxygen to the solution across the bubble surfaces and the free surface of the liquid. Across these same surfaces it continuously removed carbon dioxide and any other volatile substances that may have escaped from the seeds. Also, the mechanical action of the bubbles kept the solution in circulation above the seeds and around them and the rate of stirring was determined by the rate of bubbling. It is of course impossible to study these three effects of the bubbling gas stream separately without elaborate technique, but they need to be borne in mind in connection with all experiments with air flow.

CULTURES WITHOUT AIR FLOW

Besides the cultures that were artificially aerated by means of the air stream, there were also corresponding cultures without artificial aeration. For these the procedure was the same as that just described excepting that the flask stoppers were without tubes. Aeration of the solution was limited to such interchange between flask and chamber as might take place through the two perforations in the stopper and such very slow mixing or stirring of the solution as might be due to natural convection.

THE NUTRIENT SOLUTION AND ITS USE

The culture medium used throughout these experiments was a 3-salt nutrient solution of a type used extensively by SHIVE (24), being solution R 3.3, S 3.3 of type I, as described in the "Plan" of the Committee on the Salt Requirements of Plants, of the U. S. National Research Council (13), with equimolar partial concentrations of the three salts and a total osmotic value of about 1 atmosphere at 20°. It contained per liter 0.0084 gram-mol. of each of the salts, KH_2PO_4 , $\text{Ca}(\text{NO}_3)_2$, and MgSO_4 . No iron

was added. The salts used were of the Baker Chemical Company's "C.P., analyzed" grade. The water used was derived from a Barnstead still.

Single-salt stock solutions were prepared in a way similar to that followed by SHIVE, enough of each being prepared at the start to last throughout the whole study. The concentrations of the three stock solutions were: KH_2PO_4 , 0.59 molar; $\text{Ca}(\text{NO}_3)_2$, 1.17 molar; MgSO_4 , 1.01 molar.

Slight turbidity appeared in the KH_2PO_4 solution as originally made up and this was removed by filtering, the final concentration of the solution being ascertained by evaporating a sample to dryness, igniting the residue and weighing the latter as KPO_3 —as recommended by TREADWELL and HALL (25, p. 612). The concentration of the $\text{Ca}(\text{NO}_3)_2$ solution was ascertained gravimetrically, by precipitating the calcium of a sample as oxalate, igniting the latter and weighing it as CaO —as recommended by the authors just mentioned (25, p. 81). The MgSO_4 solution was based on the weight of salt used, considering it as $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, and no analysis of that solution was made. From the three single-salt solutions supplies of the 3-salt nutrient solution were made up in the usual way, as needed.

The nutrient solution must have altered considerably during an experiment period and it was presumably very different at the end from what it was at the start. Changes must have been brought about through absorption of water and salts by the seeds and through exudation of material from the seeds. After 24 hours at the highest temperatures used the culture solutions were noticeably turbid. The kind and amount of alteration that occurred in any flask must have been somehow a function of the combination of the variables involved, which were by nature more or less interdependent, and any attempt to study the nature and course of such solution changes adequately would be difficult.

THE SEEDS USED AND THEIR PRELIMINARY TREATMENT

The wheat seeds used in this study were of the "Nittany" variety, from a supply received from the Pennsylvania Agricultural Experiment Station in the fall of 1928. Some of this same stock had been used in MACK's study (17) of carbon-dioxide production and growth by young seedlings. No evidence has been encountered to suggest the occurrence of any alteration in the physiological characteristics of this lot of seed between the time it was received and the conclusion of the present study in the spring of 1930. It is therefore probably safe to suppose that MACK's results and those of the present study are comparable with respect to the characteristics of the seed used. The supply was stored in a burlap sack in an attic room, with temperature between 20° and about 25° . In September, 1929, weevils appeared and the entire supply was treated at that time with carbon-disulphide vapor, after which no more weevils were observed.

Sample tests made before and after this treatment showed no influence of the treatment upon the subsequent behavior of the seeds; their viability was apparently not altered at all.

The seeds for each experiment were selected with respect to specific gravity and general appearance just before they were introduced into the germination flasks. About 30 minutes before the start of an experiment approximately twice as many seeds as would be required (about 150 ml. of dry seeds) were placed in a glass pan 15 cm. in diameter and 3 cm. high and enough nutrient solution was added to just fill the pan. After vigorous stirring for a few seconds all floating seeds, comprising about 5 per cent. of the total number, were removed and the remaining seeds (those of greater specific gravity than the nutrient solution) were spread out on filter paper, from which they were counted out in 100-seed samples into 100-ml. Florence flasks, as many flasks being used as there were samples. All broken or otherwise apparently unusual seeds were rejected.

The selected seed samples were then transferred to the several germination flasks in the maintained-temperature chambers. These flasks had been prepared about an hour earlier, charged with nutrient solution, distributed among the seven temperature chambers and made ready for starting the air streams. When the seeds were introduced, the nutrient solution in each germination flask had already attained the temperature of the chamber in which it stood and the seeds must have acquired that temperature very quickly after their introduction. It required only about a minute to set the stopper in each of the germination flasks and start the air stream. The seven cultures of an experiment were started one after another in the increasing order of their temperatures and about 2 minutes elapsed in each instance. Thus the culture in the second chamber was started 2 minutes later than the one in the first chamber, the culture in the third chamber was started 4 minutes later than the one in the first, and so on, the culture in the seventh chamber being started about 12 minutes later than that in the first.

THE EXPERIMENT PERIODS

How many of the hundred seeds in any test were found to have germinated at the end of an experiment period must have depended in general on the length of the period as well as on the maintained temperature, the aeration treatment and the background conditions, the last-mentioned complex being initially alike for all experiments. The four different time periods employed in this study were 6, 12, 18 and 24 hours in length. The shorter periods were not simply observation intervals in the longest period but period length was treated as a definite experimental variable, there being a separate set of experiments for each of the four different periods. At the end of each experiment the germination flasks were re-

moved from the temperature chambers in the same order as that followed in starting the cultures. The solution was decanted and the seeds were spread out in a Petri dish, from which those that had germinated were counted to give the germination percentages. These operations required about 7 minutes in each instance; consequently the culture for 19° was observed about 7 minutes later than that for 12°, etc., and the culture for 45° was observed about 42 minutes later than that for 12°. It is evident that the actual experiment periods were not precisely the same for the different cultures of an experiment, the actual time being generally a little longer than the schedule time, with the difference somewhat greater for higher than for lower temperatures. In no instance, however, did the difference amount to more than about 30 minutes, and the nature of this study does not warrant the application of special corrections for these small discrepancies.

It may be noted that earlier students of seed germination have generally employed observation intervals as parts of a longer experiment period and that the method used in this study is comparatively new for this sort of investigation. The essential differences between these two methods of securing information on the influence exerted by the duration factor in studies of this kind deserves some attention. Let it be desired to secure experimental values for the germination percentage of a lot of seed for time periods of 6, 12, 18 and 24 hours, the environmental complex being considered as the same for all four durations, or at least for the beginnings of all tests. According to the usual procedure (as that followed in HAASIS's recent study, for instance) the cultures would all be continued for 24 hours, the number of seedlings produced at the end of 6, 12, 18 and 24 hours being ascertained by observations at the ends of the several 6-hr. intervals. After the first, second and third observations the ungerminated seeds would be returned to the standard environmental complex for the next interval. The exposure for 12 hours would consequently have to be interrupted at the end of the sixth hour, the exposure for 18 hours would have to be interrupted at the end of the sixth hour and at the end of the twelfth, and the 24-hour exposure would have to be interrupted three times, at the ends of the sixth, twelfth and eighteenth hours. Such interruptions should introduce corresponding fluctuations in the environmental complex, which usually can not be satisfactorily maintained through the short time periods requisite for observation, and these environmental fluctuations are apt to be very difficult to measure and to take into account when the experimental results come to be compared and interpreted. Common practice has been to regard the possible influence of these fluctuations as negligible, which is scarcely permissible unless suitable preliminary tests have shown this supposition to be legitimate.

When duration is treated simply as one of the experimental variables, as was done in these experiments, no interruption of exposure needs to be introduced. No culture is disturbed till the end of its own test period and observations are made only when tests have been completed. So the environmental complex is maintained throughout the experiment period without any fluctuation due to observations, though of course it may fluctuate more or less for other reasons, just as it does between times of observation when the method of intervals is employed. In the present study the relations between duration of exposure and a given set of environmental features are shown by the behavior of four different seed samples, a separate sample of 100 selected seeds being used for each of the four different exposure periods. On the other hand, the commonly used method of observation intervals and interrupted exposures does allow the same individual sample of seed to be exposed for all of the different periods considered, an advantage that is not offered by the procedure followed in this study. Various features of experimental technique, as well as the pertinent logical considerations, will naturally need to be taken into account when it is to be decided which of these two methods for dealing with the duration factor is to be employed for a projected series of experiments.

THE CRITERIA OF GERMINATION AND THE GERMINATION PERCENTAGES

For the purposes of this study a seed was considered to have germinated when the white coleoptile became visible through rupture of the seed-coat. This is the earliest stage of germination that can be readily and definitely recognized by ordinary ocular observation. At the end of each experiment each culture of 100 seeds was examined, as has been said, to ascertain how many seeds had attained this stage, including those that had progressed somewhat farther, and that number was taken as the germination percentage. Each test was repeated four times and each of the final germination-percentage values is the average of five values derived from as many like tests.

The average germination percentage for any particular environmental-durational complex indicates, of course, approximately what fraction of the number of seeds actually tested were capable of germinating with the specified complex. For example, this lot of wheat seed was of such nature that 33 per cent. of the selected seeds germinated, on the average, in 12 hours if submerged for that period under about 2 cm. of the standard nutrient solution aerated by an air flow of 1 liter per day and maintained at a temperature of 30°. No other one of the 168 different treatments tested gave this index value and only two treatments gave corresponding index values having magnitudes between 29 and 37, inclusive. Such physiological characteristics of a lot of seed may well be as valuable as other character-

istics that have been employed in describing lots of seed, such as average size, average weight, average starch percentage, etc. If a lot of seed is to be described in terms of viability (that is, capacity to produce seedlings) physiological indices of this sort will surely prove to be not only useful but in many instances quite essential. The results of this study actually furnish 168 different viability indices for the lot of seed used, based on as many different treatments or environmental-durational complexes.

It is of course also true, in a sort of converse way, that the average germination percentages resulting from a consistent and adequately inclusive set of tests are efficiency indices of the several environmental-durational complexes to which they correspond; any result of measurement defines both the thing measured and the measuring device, the former with reference to the device and the latter with reference to the thing measured. One of the aims of this study was to find out how the lot of seed used would behave or perform with reference to a series of treatments but another aim was to find out what each of the several treatments would do to representative samples of this lot of seed. Either aspect implies the other. The average percentage values are consequently to be regarded as approximate measurements of the capacity or capability of this lot of seed to produce seedlings and at the same time they are approximate measurements of the capacities of the several environmental-durational complexes to permit or call forth the production of seedlings from samples of this lot of seed. In short, we enquire, what can this lot of seed do and what can these complexes do?

As to the significance of the average germination percentages, these refer, of course, to the lot of seed used in this study and to no other lot. Other lots of the same variety or of other varieties might be expected to exhibit different kinds of germination performance. The several averages also refer to the corresponding treatments, as has just been mentioned. With another nutrient solution, with other maintained temperatures than were actually tested, or with other aeration procedures, this lot of seed or any other lot of seed might have performed very differently.

How nearly the several 100-seed samples tested may have represented the stock of seed dealt with and how nearly alike the several samples were among themselves, are important questions best answered by a study of the consistency of the whole system of numerical results. The average percentages would of course have been somewhat more nearly representative of the lot of seed from which the samples were taken if the tests had been repeated a greater number of times or if each sample had included a larger number of individual seeds. The numerical values show that there was considerable variability among the five supposedly like tests, which may indicate either effective degrees of difference between the environmental complexes for the five tests or effective differences between the capacities of the five 100-seed samples; or both sorts of differences may have occurred

together, which is not unlikely. An examination of the average percentage values leads to the conclusion that they are in general remarkably consistent and that the forms of their graphs would not have been very different if more than five tests of a kind had been made or if more than a hundred seeds had been employed in each test.

If any reader is inclined to apply statistical methods to the average germination percentages the way is clear, for all the values are given in table I. It does not appear, however, that anything would be gained by such treatment of these data and the following discussions will be based on the general consistency of the averages, which is generally of a relatively high degree for such studies as this.

GENERAL PLAN OF THE EXPERIMENTS

It was planned to cover uniformly a definite range of environmental conditions and the entire series of experiments involved tests of all possible combinations of the 7 temperatures, the 6 sets of aeration conditions and the 4 period lengths. Each experiment included just 7 cultures, one for each temperature employed but all with the same aeration treatment and the same period of incubation. The entire series of experiments involved altogether 168 different tests, each with 100 seeds that had been selected by observation and with respect to specific gravity, as has been noted. Each of the 168 tests was made five times, making a total of 840 tests. The earlier experiments were conducted singly but in the latter part of the study two experiments were conducted simultaneously. There were no indications leading to any suspicion that the lot of seed used altered in any sensible way during the term of the present study, and the general consistency of the results indicates that the experimental technique was equally satisfactory for all experiments. As a matter of record, the dates of the routine experiments are added here.

No air flow Aug. 30-Sept. 10; Oct. 8-14, 1929.
Flow of 30 l. per day Aug. 30-Sept. 10; Oct. 2-13, 1929.
Flow of 3 l. per day Oct. 13-Oct. 29, 1929.
Flow of 15 l. per day Oct. 30-Nov. 11, 1929.
Flow of 6 l. per day Nov. 12-Nov. 21, 1929.
Flow of 1 l. per day. Nov. 23-Dec. 2, 1929.

Besides the routine tests thus far described some special experiments were performed to gain information on questions that arose as the regular numerical results accumulated. A few of these will receive some attention after the routine experiments have been discussed.

Results of routine experiments

THE AVERAGE PERCENTAGES AND THE PRIMARY GRAPHS

All the percentage values from the routine experiments are presented in table I. The four main vertical sections of the table correspond to the four

TABLE I

GERMINATION PERCENTAGES FOR FIVE SEPARATE TESTS OF EACH ONE OF 168 DIFFERENT CONDITIONAL COMPLEXES, TOGETHER WITH THE AVERAGE PERCENTAGE FOR EACH COMPLEX

(FOR EACH FIVE LIKE TESTS THE MINIMUM AND THE MAXIMUM ARE IN ITALICS AND THE AVERAGE IS IN BOLDFACE TYPE)

TEMPERATURE	FOR 6-HOUR PERIOD			FOR 12-HOUR PERIOD			FOR 18-HOUR PERIOD			FOR 24-HOUR PERIOD									
	PERCENTAGES FOR 5 TESTS			AVERAGE	PERCENTAGES FOR 5 TESTS			AVERAGE	PERCENTAGES FOR 5 TESTS			AVERAGE							
Cultures without air flow	deg. C.	3	3	1	8	3	4	7	8	6	7	7	per cent.	10	13	14	14	13	per cent.
	12	5	4	4	7	11	6	15	9	10	7	13	11	17	12	21	15	14	16
	19	4	6	12	12	8	8	10	13	15	10	8	11	22	16	18	20	19	19
	24	7	7	12	11	11	10	12	16	17	14	18	*15	19	25	24	23	21	*22
	30	14	10	16	14	16	*14	15	18	17	12	13	*15	20	17	16	16	15	17
	35	10	12	15	12	13	12	16	16	12	10	12	13	13	13	13	14	16	15
	40	8	10	8	11	10	9	8	7	12	11	9	9	10	5	12	10	6	9
	45	4	4	5	3	4	4	12	7	8	7	12	9	11	14	12	7	10	11
Cultures with air flow of 1 liter per day	12	12	5	5	4	8	5	9	11	12	8	13	11	24	18	25	18	21	21
	19	12	5	9	12	12	10	15	18	16	23	22	19	63	62	56	73	53	*61
	24	11	11	10	15	10	11	35	42	29	25	37	*33	45	44	36	48	51	45
	30	13	12	16	12	10	*13	19	21	11	17	17	17	20	18	22	23	14	19
	35	10	14	11	11	15	12	11	11	9	14	13	12	14	11	16	17	9	15
	40	7	3	11	11	7	8	8	8	8	7	10	8	7	9	7	8	11	8
	45	5	3	3	5	5	4	12	7	9	9	5	8	9	10	15	12	14	12
Cultures with air flow of 3 liters per day	12	10	8	11	7	9	9	15	11	11	9	11	11	21	24	26	20	21	22
	19	13	9	13	10	11	11	21	23	18	22	20	21	58	33	45	46	46	46
	24	12	19	17	14	16	16	43	39	32	31	42	*37	45	40	67	53	55	*52
	30	18	20	14	17	15	*17	21	25	25	28	22	24	28	20	29	28	29	27
	35	16	14	16	22	16	*17	11	16	21	26	16	18	18	15	18	27	13	18
	40	13	13	11	14	14	13	15	13	12	9	17	13	16	10	14	13	10	13
	45	4	4	5	3	4	4	12	7	8	7	12	9	11	14	12	7	10	11
Cultures with air flow of 5 liters per day	12	12	5	5	4	8	5	9	11	12	8	13	11	24	18	25	18	21	21
	19	12	5	9	12	12	10	15	18	16	23	22	19	63	62	56	73	53	*61
	24	11	11	10	15	10	11	35	42	29	25	37	*33	45	44	36	48	51	45
	30	13	12	16	12	10	*13	19	21	11	17	17	17	20	18	22	23	14	19
	35	10	14	11	11	15	12	11	11	9	14	13	12	14	11	16	17	9	15
	40	7	3	11	11	7	8	8	8	8	7	10	8	7	9	7	8	11	8
	45	5	3	3	5	5	4	12	7	9	9	5	8	9	10	15	12	14	12
Cultures with air flow of 7 liters per day	12	10	8	11	7	9	9	15	11	11	9	11	11	21	24	26	20	21	22
	19	13	9	13	10	11	11	21	23	18	22	20	21	58	33	45	46	46	46
	24	12	19	17	14	16	16	43	39	32	31	42	*37	45	40	67	53	55	*52
	30	18	20	14	17	15	*17	21	25	25	28	22	24	28	20	29	28	29	27
	35	16	14	16	22	16	*17	11	16	21	26	16	18	18	15	18	27	13	18
	40	13	13	11	14	14	13	15	13	12	9	17	13	16	10	14	13	10	13
	45	4	4	5	3	4	4	12	7	8	7	12	9	11	14	12	7	10	11
Cultures with air flow of 9 liters per day	12	12	5	5	4	8	5	9	11	12	8	13	11	24	18	25	18	21	21
	19	12	5	9	12	12	10	15	18	16	23	22	19	63	62	56	73	53	*61
	24	11	11	10	15	10	11	35	42	29	25	37	*33	45	44	36	48	51	45
	30	13	12	16	12	10	*13	19	21	11	17	17	17	20	18	22	23	14	19
	35	10	14	11	11	15	12	11	11	9	14	13	12	14	11	16	17	9	15
	40	7	3	11	11	7	8	8	8	8	7	10	8	7	9	7	8	11	8
	45	5	3	3	5	5	4	12	7	9	9	5	8	9	10	15	12	14	12
Cultures with air flow of 11 liters per day	12	10	8	11	7	9	9	15	11	11	9	11	11	21	24	26	20	21	22
	19	13	9	13	10	11	11	21	23	18	22	20	21	58	33	45	46	46	46
	24	12	19	17	14	16	16	43	39	32	31	42	*37	45	40	67	53	55	*52
	30	18	20	14	17	15	*17	21	25	25	28	22	24	28	20	29	28	29	27
	35	16	14	16	22	16	*17	11	16	21	26	16	18	18	15	18	27	13	18
	40	13	13	11	14	14	13	15	13	12	9	17	13	16	10	14	13	10	13
	45	4	4	5	3	4	4	12	7	8	7	12	9	11	14	12	7	10	11
Cultures with air flow of 13 liters per day	12	12	5	5	4	8	5	9	11	12	8	13	11	24	18	25	18	21	21
	19	12	5	9	12	12	10	15	18	16	23	22	19	63	62	56	73	53	*61
	24	11	11	10	15	10	11	35	42	29	25	37	*33	45	44	36	48	51	45
	30	13	12	16	12	10	*13	19	21	11	17	17	17	20	18	22	23	14	19
	35	10	14	11	11	15	12	11	11	9	14	13	12	14	11	16	17	9	15
	40	7	3	11	11	7	8	8	8	8	7	10	8	7	9	7	8	11	8
	45	5	3	3	5	5	4	12	7	9	9	5	8	9	10	15	12	14	12
Cultures with air flow of 15 liters per day	12	10	8	11	7	9	9	15	11	11	9	11	11	21	24	26	20	21	22
	19	13	9	13	10	11	11	21	23	18	22	20	21	58	33	45	46	46	46
	24	12	19	17	14	16	16	43	39	32	31	42	*37	45	40	67	53	55	*52
	30	18	20	14	17	15	*17	21	25	25	28	22	24	28	20	29	28	29	27
	35	16	14	16	22	16	*17	11	16	21	26	16	18	18	15	18	27	13	18
	40	13	13	11	14	14	13	15	13	12	9	17	13	16	10	14	13	10	13
	45	4	4	5	3	4	4	12	7	8	7	12	9	11	14	12	7	10	11
Cultures with air flow of 17 liters per day	12	12	5	5	4	8	5	9	11	12	8	13	11	24	18	25	18	21	21
	19	12	5	9	12	12	10	15	18	16	23	22	19	63	62	56	73	53	*61
	24	11	11	10	15	10	11	35	42	29	25	37	*33	45	44	36	48	51	45
	30	13	12	16	12	10	*13	19	21	11	17	17	17	20	18	22	23	14	19
	35	10	14	11	11	15	12	11	11	9	14	13	12	14	11	16	17	9	15
	40	7	3	11	11	7	8	8	8	8	7	10	8	7	9	7	8	11	8
	45	5	3	3	5	5	4	12	7	9	9	5	8	9	10	15	12	14	12
Cultures with air flow of 19 liters per day	12	10	8	11	7	9	9	15	11	11	9	11	11	21	24	26	20	21	22
	19	13	9	13	10	11	11	21	23	18	22	20	21	58	33	45	46	46	46
	24	12	19	17	14	16	16	43	39	32	31	42	*37	45	40	67	53	55	*52
	30	18	20	14	17	15	*17	21	25	25	28	22	24	28	20	29	28	29	27
	35	16	14	16	22	16	*17	11	16	21	26	16	18	18	15	18	27	13	18
	40	13	13	11	14	14	13	15	13	12	9	17	13	16	10	14	13	10	13
	45	4	4	5	3	4	4	12	7	8	7	12	9	11	14	12	7	10	11
Cultures with air flow of 21 liters per day	12	12	5	5	4	8	5	9	11	12	8	13	11	24	18	25	18	21	21
	19	12	5	9	12	12	10	15	18	16	23	22	19	63	62	56	73	53	*61
	24	11	11	10	15	10	11	35	42	29	25	37	*33	45	44	36	48	51	45
	30	13	12	16	12	10	*13	19	21	11	17	17	17	20	18	22	23	14	19
	35	10	14	11	11	15	12	11	11	9	14	13	12	14	11	16	17	9	15
	40	7	3	11	11	7	8	8	8	8	7	10	8	7	9	7	8	11	8
	45	5	3	3	5	5	4	12	7	9	9	5	8	9	10	15	12	14	12
Cultures with air flow of 23 liters per day	12	10	8	11	7	9	9	15	11	11	9	11	11	21	24	26	20	21	22
	19	13	9	13	10	11	11	21	23	18	22	20	21	58	33	45	46	46	46
	24	12	19	17	14	16	16	43	39	32	31	42	*37	45	40	67	53	55	*52
	30	18	20	14</															

TABLE I (continued)

TEMPERATURE	FOR 6-HOUR PERIOD			FOR 12-HOUR PERIOD			FOR 18-HOUR PERIOD			FOR 24-HOUR PERIOD		
	PERCENTAGES FOR 5 TESTS		AVERAGE	PERCENTAGES FOR 5 TESTS		AVERAGE	PERCENTAGES FOR 5 TESTS		AVERAGE	PERCENTAGES FOR 5 TESTS		AVERAGE
Cultures with air flow of 6 liters per day	5 3 6 3 3		per cent.	9 7 9 7 8		8	19 21 13 15 19		per cent.	27 26 30 15 25		per cent.
	5 10 7 16 8		4	19 20 23 16 16		20	27 45 39 28 27		17	68 74 66 65 77		25
	8 15 15 8 8		9	45 38 39 39 38		20	83 77 78 75 79		33	90 90 94 91 95		70
	12 10 11 12 18		11	63 49 53 53 66		*58	67 67 65 65 60		*78	80 73 79 79 65		*92
	18 21 17 24 15		14	26 28 29 27 30		*58	27 27 33 28 24		66	32 28 32 29 31		75
	24 15 22 19 10		*19	21 26 23 18 21		28	21 24 23 19 19		28	20 29 17 19 27		30
Cultures with air flow of 15 liters per day	27 8 12 14 11		18	19 16 13 12 11		22	16 10 12 15 16		22	13 21 14 14 12		22
	3 3 7 3 4		14	11 10 7 9 7		14	10 13 18 17 12		14	30 36 29 22 29		15
	9 8 10 6 5		4	12 22 23 19 10		9	29 38 50 55 50		14	90 80 62 71 76		29
	17 15 19 13 10		8	44 42 43 43 43		17	91 70 83 95 85		44	90 99 86 93 99		76
	22 20 16 20 16		15	68 54 67 56 53		43	90 85 85 75 79		*83	90 84 88 80 79		*96
	20 23 19 21 19		19	35 22 31 28 32		*60	43 30 25 27 31		*83	36 38 31 30 31		84
Cultures with air flow of 30 liters per day	26 20 21 17 17		*20	25 24 33 15 20		30	19 22 22 26 22		31	17 20 22 32 22		33
	5 15 19 18 17		*20	9 19 30 14 12		22	16 15 24 11 11		22	10 11 20 22 32		22
	4 3 3 2 3		15	6 10 19 8 16		15	16 22 12 17 12		15	31 35 35 22 23		15
	9 6 9 9 4		3	22 19 27 22 24		12	45 66 57 39 46		16	94 92 86 71 65		29
	12 7 8 12 11		7	57 59 41 30 42		23	97 92 91 81 59		51	99 100 95 91 89		82
	19 17 17 20 12		10	70 81 78 64 75		46	90 88 91 80 79		84	99 94 95 93 87		*96
Cultures with air flow of 60 liters per day	17 13 21 19 24		*19	29 34 19 32 28		*74	44 22 33 44 35		*86	53 35 34 49 55		93
	20 22 15 12 24		*19	23 23 20 20 14		28	20 21 23 20 18		36	27 22 21 19 15		45
	11 18 15 17 16		15	14 14 17 18 12		20	13 16 17 12 17		20	11 19 16 13 14		21

Maximum average.

periods used and the six horizontal sections represent the different aeration treatments. Each group of 35 values thus represents a single experiment performed five times. For each time an experiment was performed there are 7 percentage values, corresponding to the seven different maintained temperatures. The minimum and the maximum in each horizontal series of five values from like tests are in italics and the average of each five corresponding values is shown in boldface type. There are 168 of these averages. The highest average for each experiment is marked by an asterisk. Of course the temperature corresponding to this highest average represents about the optimal temperature for seedling production in the experiment in question. For some experiments there are two highest averages, of the same magnitude, which of course implies that the optimal temperature for that experiment may be considered as lying somewhere between the two temperatures thus indicated, or else slightly below the lower one or slightly above the higher one.

It may be noted that most low percentage values may be considered as relatively less precise than high ones. To be counted as germinated at the end of any test a seed must have burst its seedcoat, though some additional growth may have occurred. At the end of an 18-hr. or 24-hr. period with good conditions for germination many seedlings had elongated considerably, i.e., their seeds had passed the critical moment of bursting the seedcoats. But no account is taken in table I of this over-growth, since whenever over-growth occurred there are data for one or more shorter periods. Thus, for example, the average percentage value of 67 for 30°, 3 l. and 24 hr. obviously includes the seeds that had germinated in the 18-hr., 12-hr. and 6-hr. periods. But the value (16) for the 6-hr. period under these conditions represents no considerable over-growth. Such considerations are clearly cared for if 6-hr. increments of the average percentages are computed by subtraction and that mode of presentation will be employed below (table II).

On the other hand, at the end of any period a seed might not yet have burst its seedcoat, being consequently counted as ungerminated, but the processes leading to germination might have progressed very far; such a seed might have been recorded as germinated if the period had been an hour or two longer. As the percentage value is higher there is less probability of nearly-germinated seeds, since the maximum possible value in any case can not be above 100. In any case this lack of precision can never amount to more than a few units of the average percentage value. Furthermore, it is not to be considered at all in instances where the next longer period showed no increase; for example, the value 27, for 35°, 3 l. and 18 hr. can not lack precision in this respect because the corresponding 24-hr. period shows exactly the same average percentage as is shown by this 18-hr. period.

It is at once apparent from table I that, within the ranges dealt with, all three experimental variables (maintained temperature, aeration treatment and duration of incubation) were influential in determining the percentages and their averages. The *lowest germination percentages* correspond to environmental complexes that had either low or high temperatures, low degrees of aeration or short periods, or two of these features or all three of them together. The actual minimum percentage is 1 (for the third test of 12° without air flow and with a 6-hr. incubation period) and the lowest average percentage is 3 (for 12°, 6 hr. and air flow of 30 l.). Averages below 5 represent all the 6-hr. tests with 12° and all other tests have averages of 5 or above. Averages of 10 or below may be taken as representing the least efficient environmental complexes studied. These are confined to the following combinations: All 6-hr. tests with 12° and all 12-hr. tests with that temperature, excepting the one with air flow of 30 l. (which gave an average of only 12); all 6-hr. tests with 19°; all tests with 24° and without air flow or with air flow of 1 l. or 30 l.; and all 6-hr., 12-hr., 18-hr. and 24-hr. tests with 45° and without air flow or with air flow of 1 l.

The *highest germination percentages* correspond to combinations of 24° or 30° with 18-hr. or 24-hr. periods and air flow of 6 l., 15 l., or 30 l. Average values above 90 are confined to the 24-hr. tests with 24° and air flow of 6 l., 15 l., or 30 l., and with 30° and air flow of 30 l.

THE TEMPERATURE GRAPHS

Many details concerning the relations of the various environmental combinations included in this study are most readily brought out by means of graphs. The average percentages shown in boldface type in table I may be plotted in many ways, only a few of which will be considered in detail here. Attention will be confined to the graphs on which the percentages are plotted as ordinates. These are of three sorts: (A) those on which temperature values are abscissas (24 graphs), (B) those on which aeration indices are abscissas (28 graphs) and (C) those on which the time indices are abscissas (42 graphs). Each of these three groups of graphs may be arranged for convenient study in two ways. For the first group the 24 temperature graphs may be arranged (A 1) in 6 sheaves of 4 graphs each, every sheaf representing a single aeration treatment while each individual graph represents a single length of incubation period; or they may be arranged (A 2) in 4 sheaves (for the incubation periods) of 6 graphs each (for the aeration treatments). The 28 aeration graphs may be assembled (B 1) in 7 sheaves (for the temperatures) of 4 graphs each (for the times of incubation), or (B 2) in 4 sheaves (times of incubation) of 7 graphs each (temperatures). In like manner the 42 duration graphs may be assembled (C 1) in 7 sheaves (temperatures) of 6 graphs each (aeration treat-

ments) or (C 2) in 6 sheaves (aeration treatments) of 7 graphs each (temperatures). All of these sets of graphs may be readily constructed from the data given in table I. A few of them are presented here, in figures 1-4.

Figure 1 gives representative samples of arrangement A 1. Three of the six sheaves are shown, for cultures without air flow and with air flow of

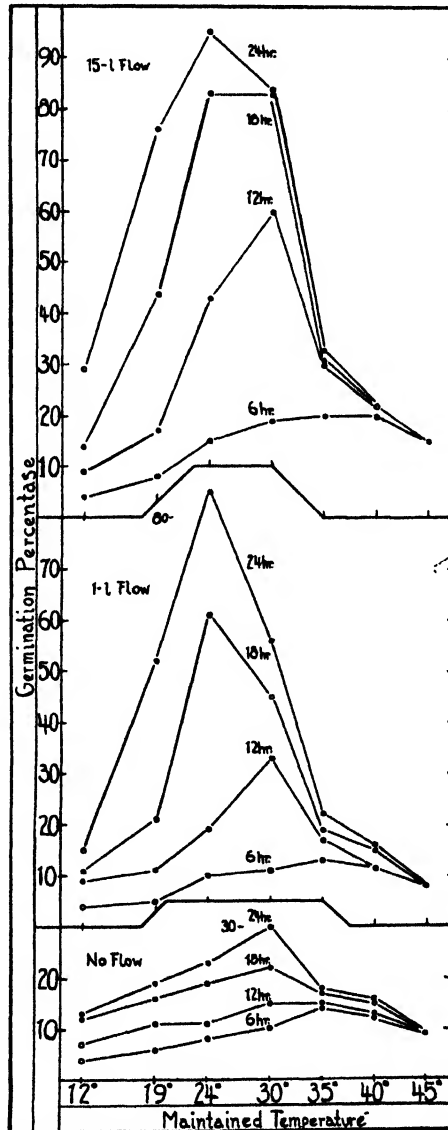


FIG. 1. Representative temperature graphs of germination percentage, grouped according to aeration.

1 l. and 15 l. per day. Each sheaf comprises four individual graphs, one for each of the four incubation periods.

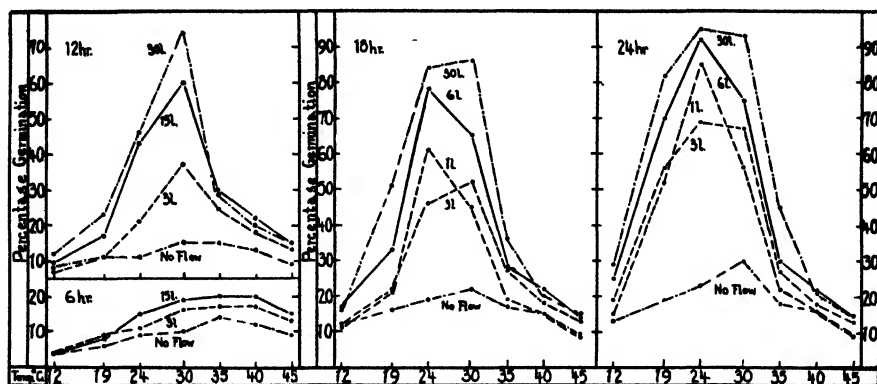


FIG. 2. Representative temperature graphs of germination percentage grouped according to length of incubation period. ("Percentage germination" should be *germination percentage*.)

Figure 2 presents arrangement A 2, with four sheaves, one for each incubation period. For the sake of clearness one or more graphs are omitted from each sheaf but the general relations are adequately shown.

Some features brought out by these temperature graphs will now be mentioned, reference being made to table I and figures 1 and 2. It is seen that the form of the temperature graph differs markedly according to period length and aeration treatment. All of the 24 graphs agree, as would be expected, in showing relatively low percentage values for 12° and for 45°, with relatively higher values for the intervening temperatures. No minimal nor maximal temperature for germination is shown but the percentages for all tests with 6-hr. period and a temperature of 12° closely approach zero, which suggests that the minimal temperature for germination in 6 hours must have been not far below 12°. For the longer incubation periods the percentages for 12° are progressively higher (for the 24-hr. period, more divergent) and it is suggested that the corresponding minimal temperatures may have been increasingly below 12° with progressively more vigorous aerations as well as with progressively longer periods. The temperature minimum for seedling production by this lot of wheat seed may consequently be said to have been more or less below 12° for all tests, probably farther below that temperature for the tests with longer periods, especially with the more vigorous aeration treatments. For ready comparison, all the average percentages for 12° are shown on page 227.

For the few seeds that germinated at 12° in 6 hr. the aeration differences were apparently without influence, but for those that germinated in longer

	FOR 6-HR. PERIOD, 12°	FOR 12-HR. PERIOD, 12°	FOR 18-HR. PERIOD, 12°	FOR 24-HR. PERIOD, 12°
No flow	4	7	12	13
1-l. flow	4	9	11	15
3-l. flow	4	8	12	19
6-l. flow	4	8	17	25
15-l. flow	4	9	14	29
30-l. flow	3	12	16	29

periods the more vigorous aeration treatments gave appreciably higher percentages. For each aeration treatment the longer the period of incubation the higher is the germination percentage, as would be expected. But the highest germination percentage obtained for 12° was only 29 (for 24 hr. and air flow of 15 l. and 30 l.). It is apparent that no considerably higher percentages than those given would have been obtained for a temperature of 12° combined with any one of these four incubation periods if the air flow had been still more rapid. But it is highly probable that any 24-hr. value for 12° would have been notably surpassed with any one of these aeration treatments if the incubation period had been sufficiently longer than 24-hr.

Turning to the germination percentages shown for the highest temperature employed (45°), all four periods gave the same percentage of germination with all aeration treatments, which indicates that no additional seeds germinated at 45° after the first 6 hr. of incubation, no matter what aeration treatment was employed. It is not likely that a period longer than 24 hr. would have given any additional germination with this temperature for any aeration treatment in the range of treatments studied. This temperature was surely supra-optimal and consequently probably increasingly injurious as time elapsed; those seeds that could germinate quickly under these conditions might do so but those that were delayed more than 6 hr. were unable to germinate at all. This thought is the same as was suggested by HAASIS (7) for a similar state of affairs with his conifer seeds. Although differences in period length did not influence the germination percentage for 45° with any aeration treatment tested, as has just been noted, it is remarkable that the percentage value for this highest temperature combined with any length of period is higher for air flows of 3 l., 6 l., 15 l. or 30 l. than for no flow or for a flow of only 1 l. per day. All the percentage values for 45° are shown on the following page, for comparison among themselves and with those for 12°, given above.

It is indicated that the maximal temperature for germination of this lot of seed was always considerably above 45°, apparently somewhat farther above that temperature with air flow of 3 l. or more but apparently not

	FOR 6-HR. PERIOD, 45°	FOR 12-HR. PERIOD, 45°	FOR 18-HR. PERIOD, 45°	FOR 24-HR. PERIOD, 45°
No flow	9	9	9	9
1-l. flow	8	8	8	8
3-l. flow	13	13	13	13
6-l. flow	14	14	14	15
15-l. flow	15	15	15	15
30-l. flow	15	15	15	15

any higher, for any aeration treatment, with an incubation period of 12, 18 or 24 hr. than with a period of only 6 hr.

With regard to the intermediate temperatures tested (19°, 24°, 30°, 35°, and 40°), all the temperature graphs have the usual form, with a generally well defined graph maximum, the abscissa of which is to be taken as about the temperature optimum in each instance. For any aeration treatment this maximum is always progressively greater with longer periods (fig. 1), as might be expected, and it is generally greater for more vigorous than for less vigorous aeration (fig. 2). The lowest value of this graph maximum is 13 or 14 per cent. (for 6 hr. and 35°, with no flow or a flow of 1 l. per day) and its highest value is 92 or 95 per cent. (for 24 hr. and 24°, with air flow of 15 l. or 30 l. per day). The values for all temperature-graph maxima (maximal ordinates) are shown below, the corresponding abscissa value (about the optimal temperature) being shown in parenthesis in each case.

	GRAPH MAXIMUM FOR 6-HR. PERIOD	GRAPH MAXIMUM FOR 12-HR. PERIOD	GRAPH MAXIMUM FOR 18-HR. PERIOD	GRAPH MAXIMUM FOR 24-HR. PERIOD
No flow	14 (35°)	15 (30°, 35°)	22 (30°)	30 (30°)
1-l. flow	13 (35°)	33 (30°)	*61 (24°)	*85 (24°)
3-l. flow	17 (30°, 35°)	37 (30°)	*52 (30°)	*69 (24°)
6-l. flow	19 (30°)	58 (30°)	78 (24°)	92 (24°)
15-l. flow	20 (35°, 40°)	60 (30°)	83 (24°, 30°)	95 (24°)
30-l. flow	19 (35°, 40°)	74 (30°)	86 (30°)	95 (24°)

As to the general relation of aeration treatment to the maximal percentage value for any length of period, there are four exceptions to the statement that the maximum is greater for more vigorous than for less vigorous aeration: (1) the 6-hr. maximum for a flow of 1 l. (13 per cent.) is not greater than that for no air flow (14 per cent.); (2) the 6-hr. maximum for a 30-l. flow (19 per cent.) is not greater than that for a 15-l. flow (20 per cent.); (3) the 18-hr. maximum for a 3-l. flow (52 per cent.) is

clearly smaller than that for a 1-l. flow (61 per cent.); and (4) the 24-hr. maximum for a 3-l. flow (69 per cent.) is much smaller than that for a 1-l. flow (85 per cent.). The first two of these exceptions may be regarded as not significant but the last two appear to be worthy of careful consideration and their values are marked with asterisks in the above tabulation. They will be reverted to later.

One of the most striking features of the temperature relations here in question is the regression of the optimal temperature with increasing length of the incubation period, for any aeration treatment (fig. 1). It is not quite regular, but there appears to be no doubt of its significance. Other conditions being nearly alike, the optimal temperature for the production of seedlings by this lot of seed is generally lower for longer incubation. If the whole range of period lengths dealt with is considered there are no exceptions to this and the total regression is from about 30° or 35° (or even higher) to about 24°.

A similar regression of the optimal temperature for germination was reported by HAASIS for his conifer seeds. In some cases, as has been said, he found also a double temperature optimum when the length of the incubation period was properly chosen, other influences being the same and favorable to germination, but no indication of a double temperature optimum (bimodal temperature curve) was observed in the present study.

A change in temperature optimum with lapse of time is apparently generally characteristic of growth rates, as has been pointed out by LEHENBAUER (11), TALMA (quoted by BENECKE-JOST, 2, p. 37), FAWCETT (5) and GERICKE (6); but it does not appear to have been emphasized for germination percentage (which is of course fundamentally different from growth rate) excepting by HAASIS. The general question of growth retardation with the lapse of time, when temperature and other influences are maintained, was discussed with characteristic acumen by F. F. BLACKMAN (3).

TEMPERATURE OPTIMA BASED ON AVERAGE HOURLY RATES OF SEEDLING PRODUCTION

To compute the average hourly rate of seedling production corresponding to the highest percentage value for each combination of aeration treatment and duration of incubation we divide each of the maximal percentages by the length of the period that gave it. For example, 6 hr. of a 6-l. air flow gave a maximal germination percentage of 19 (30°) and an average rate of 3.2 seedlings per hour; 12 hr. of a 30-l. flow gave a maximal percentage of 74 (30°) and an average rate of 6.2 seedlings per hour; etc. The average hourly rate of maximal seedling production for all the combinations of aeration treatment and duration are shown on page 230, with the corresponding temperatures in parenthesis.

	HIGHEST AVERAGE HOURLY RATES FOR 6-HR. PERIOD	HIGHEST AVERAGE HOURLY RATES FOR 12-HR. PERIOD	HIGHEST AVERAGE HOURLY RATES FOR 18-HR. PERIOD	HIGHEST AVERAGE HOURLY RATES FOR 24-HR. PERIOD
No flow	2.3 (35°)	1.3 (30°, 35°)	1.2 (30°)	1.3 (30°)
1-l. flow	2.2 (35°)	2.8 (30°)	3.4 (24°)	3.5 (24°)
3-l. flow	2.8 (30°, 35°)	3.1 (30°)	2.9 (30°)	2.9 (24°)
6-l. flow	3.2 (30°)	4.8 (30°)	4.3 (24°)	3.8 (24°)
15-l. flow	3.3 (35°, 40°)	5.0 (30°)	4.6 (24°, 30°)	4.0 (24°)
30-l. flow	3.2 (35°, 40°)	6.2 (30°)	4.8 (30°)	4.0 (24°)

It is seen that this highest average hourly rate was, for each period length, significantly greater with air flow of 6 l., 15 l. or 30 l. than with less vigorous aeration treatments and that for all period lengths excepting the shortest one it was significantly greater with air flow of 1 l. or 3 l. than without any air flow. Furthermore, with air flows of 6 l., 15 l. and 30 l., this index of efficiency has its greatest magnitude (4.8, 5.0, 6.2) for the 12-hr. period and its least magnitude (3.2, 3.3, 3.2) for the 6-hr. period; but this magnitude is about the same (2.8, 3.1, 2.9, 2.9) for all four periods with a flow of 3 l., while it is greatest for the 18-hr. and 24-hr. periods (3.4, 3.5) with a flow of 1 l. Without air flow it is greatest (2.3) for the shortest period.

With regard to average hourly rates the optimal combination of temperature, aeration treatment and time is shown as that for 30°, 30-l. flow and 12 hr., which environmental combination gave the hourly rate of 6.2. The next to the highest rate is 5.0 (for 30°, 15-l. flow, 12 hr.) and a rate of 4.8 is shown alike for the combination 30°, 6-l. flow, 12 hr., and for the combination 30°, 30-l. flow and 12 hr. The highest hourly rate shown for 24° in any combination is very much lower than the ones for 30°; it is 4.6 for the combination of 24°, 15-l. flow, 18 hr., but this same rate is given for the combination 30°, 15-l. flow, 18 hr. and the highest 24°-rate not equalled or surpassed by some 30°-rate is only 4.3, for the combination 24°, 6-l. flow, 18 hr. The optimal temperature for average hourly production of seedlings is clearly 30° and not 24°.

THE AERATION GRAPHS

Figure 3 presents aeration graphs of germination percentage for the 6-hr. and the 24-hr. incubation periods. They are representative of the group of 42 graphs previously referred to as B 2. The graphs for 12°, 19°, 24°, and 30° are continuous lines and those for 35°, 40°, and 45° are broken lines. To save space, two sections of this figure have been cut out, as is indicated, but all points of observation are shown.

The percentage values for the 6-hr. period are all low and about all that can be said of them in general is that more vigorous aeration treatment for

any temperature usually shows somewhat higher percentages, up to an air flow of 6 l. per day, while still more rapid rates of air flow show no significantly greater percentages. No influence of aeration treatment is evident for the 6-hr. period and 12°. For the 6-hr. period and 24° an optimal air flow appears as 15 l. per day and it is suggested that the 30-l. flow may have been supraoptimal for 30° and 35° as well as for 24°. For the 12-hr.

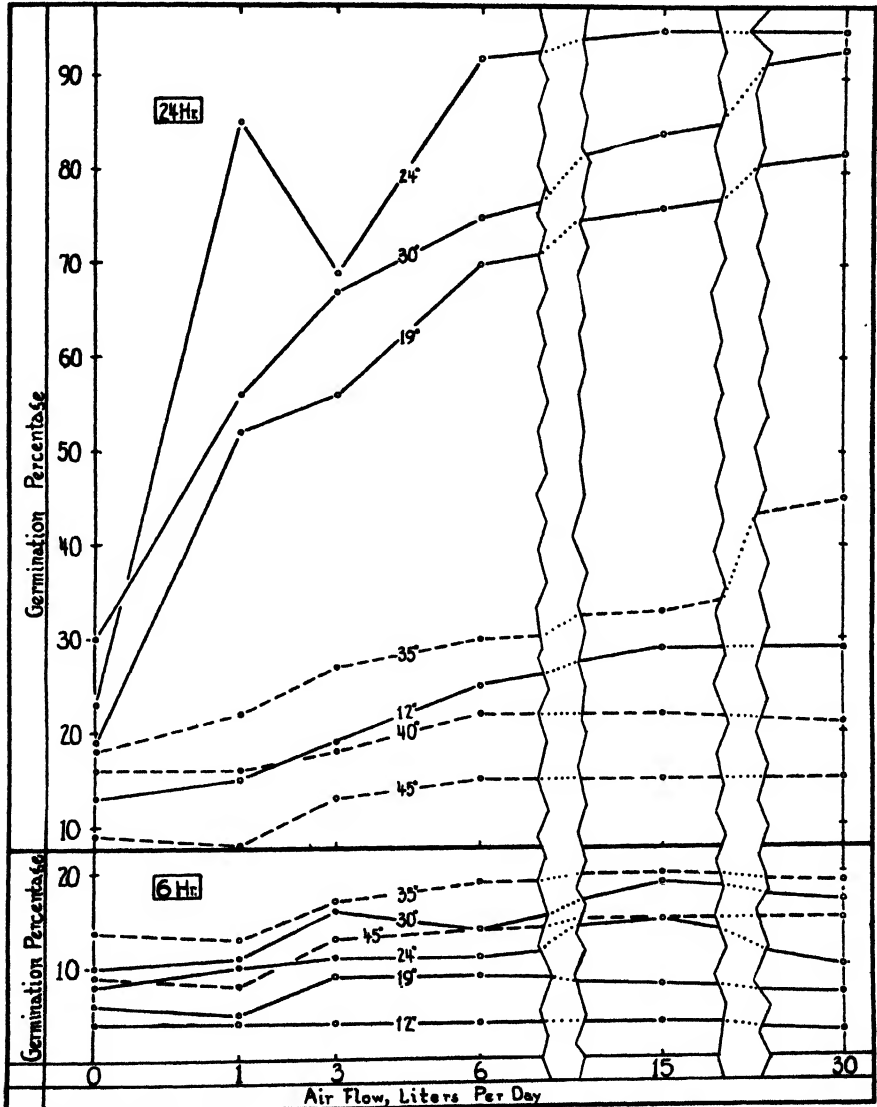


FIG. 3. Representative aeration graphs of germination percentage, for 6 hr. and for 24 hr. of incubation. Two portions are cut out to save space.

period (for which no graphs are shown) no optimal aeration treatment appears for 24° or 30°, and a flow of 30 l. per day gave percentages for the other temperatures just as high as were given for a flow of 6 l. For the 18-hr. period (graphs not shown) the temperatures for which no aeration optimum was attained are 19° and 35°; the 6-l. flow gave as high values as the 30-l. flow for 40° and 45° and the 15-l. flow gave as high values as the 30-l. flow for 24° and 30°.

The sheaf of graphs for the 24-hr. period (figure 3) deserves special attention. They are not very different from those for the 18-hr. period, which are not shown here. The average germination percentage is seen to be generally higher with more vigorous aeration, but for temperatures of 40° and 45° this is not true for air flows more rapid than 6 l. per day, and for temperatures of 12° and 24° the percentage value for the 30-l. air flow is not greater than that for the 15-l. flow. The most consistently upward-sloping graphs are those for 19°, 30° and 35° and for these three temperatures some rate of air flow greater than 30 l. per day might have given a percentage value still higher than the highest one here shown. This suggests that, for these three temperatures, the optimal rate of air flow was not attained, though it appears to have been attained for 12°, 24°, 40° and 45°, within the ranges of conditions here studied. The general air-flow optimum is seen to be the range from 6 l. to 30 l. and any more rapid flow could not have given a percentage much above 93 or 95, since these values closely approach the fixed limit of 100 per cent.

The 24-hr. aeration graph for 24° is remarkable for an apparent secondary minimal point for the 3-l. air flow, which has been mentioned above. This kind of irregularity is definitely shown only for 24 hr., 3 l. (fig. 3) and for 18 hr., 24°, 3 l., but it is suggested by the forms of the graphs for 24 hr., 19°, 3 l., for 18 hr., 19°, 3 l. and perhaps for 12 hr., 30°, 3 l.

This secondary minimal point is of special interest in connection with the double oxygen optima recently reported by MACK (17) for carbon-dioxide production and shoot elongation in wheat seedlings from the same lot of seed as was used in this study. In the present case it appears that, with a properly chosen maintained temperature (about 24°, which is near the general temperature optimum for this lot of seed) an air flow of 3 l. per day gave a markedly lower germination percentage in 18 hr. or 24 hr. than was given by either less vigorous or more vigorous aeration with the same periods of incubation.

These observations on aeration optima may be important when we shall have learned more of the oxygen requirements of germinating seeds. For many kinds of seed oxygen supply appears to be just as truly important and critical as temperature and water supply are, and aeration of such cultures as those of the present study may be effective—as has been re-

marked—in other ways than through its influence on oxygen supply. To secure very high germination percentages (approaching 100) in 24 hr. or 18 hr., with this lot of seed under dilute nutrient solution, it was only necessary to find the correct combination of temperature, aeration treatment and length of incubation period.

THREE-DIMENSION GRAPHS

Another way to bring the average percentages of table I together for somewhat ready comparison, and to show how markedly different environmental complexes gave similar results, is to plot the percentages on three-dimensional diagrams, with contour lines or isopleths connecting points of like value. Since there are four variables to be dealt with and only three may be plotted on a single diagram, several diagrams are requisite to represent all the relations. If we consider the diagrams as representing curved surfaces and always plot the average percentages as altitudes, three sets of diagrams are possible. These may be characterized as follows, the terminology being that of a topographic chart.

SET A.—West-east distances represent the different degrees of aeration treatment and south-north distances represent temperature. There are four diagrams in this set, one for each incubation period. The set for 24 hr. is shown in figure 4 (A).

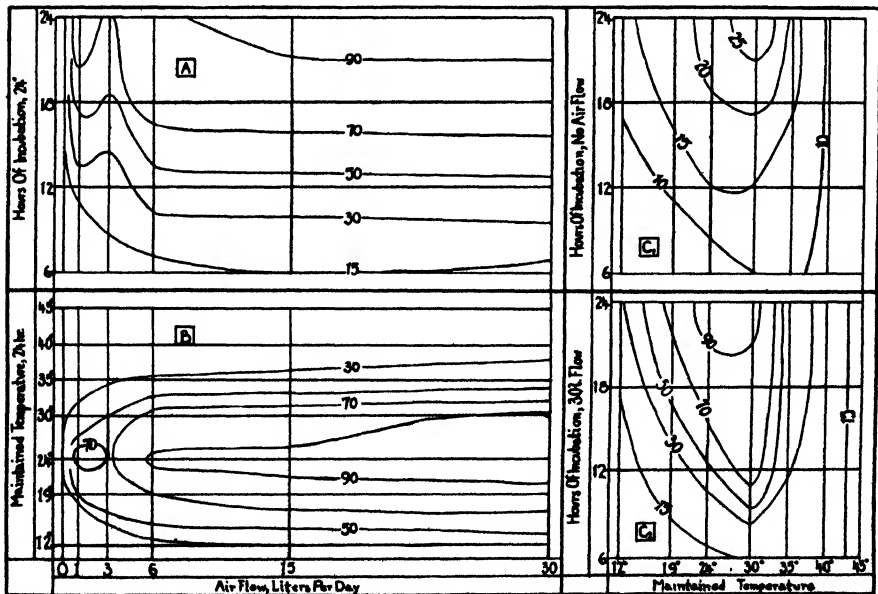


FIG. 4 Representative three-dimension graphs of germination percentage as related to maintained temperature, aeration treatment and duration of incubation.

SET B.—West-east distances represent aeration treatments, as in set A, but south-north distances represent different incubation periods. In this set there are seven diagrams, one for each temperature. The set for 24° is shown in figure 4 (B).

SET. C.—West-east distances represent temperature and south-north distances represent, as in set B, the different incubation periods. There are six diagrams, one for each aeration treatment. The sets for no air flow and for a flow of 30 l. per day are shown in figure 4 (C_1 and C_2).

Each isopleth or contour line of each diagram passes through points of like altitude and all points on any line consequently represent conditional complexes giving about the same average germination percentage. Thus each diagram is seen to be divided into areas by the contours, like a physiographic chart of a terrain. The percentage values are shown on the contours. Each diagram has a "high" and a "low" area and several intermediate ones.

These isopleth diagrams bring out the relations already mentioned and many others in addition, but they need not be discussed in detail. This method of presentation of the numerical values shows that the same percentage value was given by many different treatments of the cultures and that different but equally effective treatments are consistently related to the system of coordinates.

SIX-HOUR AND TWELVE-HOUR INCREMENTS OF THE AVERAGE
PERCENTAGE VALUES FOR THE TWENTY-FOUR HOURS
AFTER THE BEGINNING OF THE EXPERIMENTS

If we consider that the number of germinated seeds in any culture increased with time we may derive 6-hr. and 12-hr. increments by the ordinary method of subtraction. The results of these operations are set forth in table II, which consists of six horizontal sections, one for each aeration treatment. In each section are seven lines, one for each of the seven temperatures, and each line presents, in order from left to right, the increments for the first, second, third and fourth 6-hr. periods, for the first and second 12-hr. periods and the total percentage value for the entire 24-hr. period. The highest value for each period in each section of the table is shown in boldface type and the highest 6-hr. value for each aeration treatment is designated by an asterisk.

The temperature giving the greatest 6-hr. increment value with any air flow is progressively lower for later periods. For example, with air flow of 15 l. per day the optimal temperature for seedling production in the first 6 hours is shown as 35° or 40°, for the second 6-hr. period it is 30°, for the third it is 24° and for the fourth it is 19°. This relation holds for all air flows tested but does not appear to hold for the cultures without air flow.

TABLE II
SIX-HOUR AND TWELVE-HOUR INCREMENTS OF THE AVERAGE PERCENTAGES SHOWN IN TABLE I

AERATION	TEMPERATURE	For 1st 6 HRS.	For 2ND 6 HRS.	For 3RD 6 HRS.	For 4TH 6 HRS.	For 1st. 12 HRS.	For 2ND 12 HRS.	For 1st 24 HRS.
Cultures without air flow	<i>deg. C.</i>							
	12	4	3	5	1	7	6	13
	19	6	5	5	3	11	8	19
	24	8	3	8	4	11	12	23
	30	10	5	7	8	15	15	30
	35	*14	1	2	1	15	3	18
	40	12	1	2	1	13	3	16
	45	9	0	0	0	9	0	9
Cultures with air flow of 1 l. per day	12	4	5	2	4	9	6	15
	19	5	6	10	31	11	41	52
	24	10	9	*42	24	19	66	85
	30	11	22	12	11	33	23	56
	35	13	4	2	3	17	5	22
	40	12	0	3	1	12	4	16
	45	8	0	0	0	8	0	8
Cultures with air flow of 3 l. per day	12	4	4	4	7	8	11	19
	19	9	2	11	*34	11	45	56
	24	11	10	25	23	21	48	69
	30	16	21	15	15	37	30	67
	35	17	7	3	0	24	3	27
	40	17	1	0	0	18	0	18
	45	13	0	0	0	13	0	13

* Highest 6-hr. value for the given aeration treatment.

TABLE II (continued)

AERATION	TEMPERATURE	FOR 1ST 6 HRS.	FOR 2ND 6 HRS.	FOR 3RD 6 HRS.	FOR 4TH 6 HRS.	FOR 1ST 12 HRS.	FOR 2ND 12 HRS.	FOR 1ST 24 HRS.
Cultures with air flow of 6 l. per day	deg. C.							
	12	4	4	9	8	8	17	25
	19	9	11	13	37	20	50	70
	24	11	29	38	14	40	52	92
	30	14	*44	7	10	58	17	75
	35	19	9	0	2	28	2	30
	40	18	4	0	0	22	0	22
	45	14	0	0	1	14	1	15
Cultures with air flow of 15 l. per day	12	4	5	5	15	9	20	29
	19	8	9	27	32	17	59	76
	24	15	28	40	12	43	52	95
	30	19	*41	23	1	60	24	84
	35	20	10	1	2	30	3	33
	40	20	2	0	0	22	0	22
	45	15	0	0	0	15	0	15
Cultures with air flow of 30 l. per day	12	3	9	4	13	12	17	29
	19	7	16	28	31	23	59	82
	24	10	36	38	11	46	49	95
	30	17	*57	12	7	74	19	93
	35	19	9	8	9	28	17	45
	40	19	1	0	1	20	1	21
	45	15	0	0	0	15	0	15

* Highest 6-hr. value for the given aeration treatment.

Of course this generalization constitutes a step in the analysis of the reasons for the temperature regression already noted.

If we arrange the 6-hr.-increment values that are above 30 per cent. in the descending order of their magnitudes we obtain the following list. Opposite each increment value is shown the particular combination of temperature and air flow that gave it and the number of the 6-hr. period for which it occurs. With the single exception of the value 36, every one of

6-HR. INCREMENT	TEMPERATURE	RATE OF AIR FLOW	NO. OF PERIOD
<i>per cent.</i>	<i>deg. C.</i>	<i>l. per day</i>	
57	30	30	2
44	30	6	2
42	24	1	3
41	30	15	2
40	24	15	3
38	24	30.6	3
37	19	6	4
36	24	34	2
34	19	3	4
32	19	15	4
31	19	1, 30	4

these corresponds to one or another of the three temperature-period combinations, 30°, 2nd period; 24°, 3rd period; and 19°, 4th period. The aeration relations of these best combinations are shown by the upper three graphs of figure 5. In that figure abscissas are aeration treatments and ordinates are 6-hr. germination-percentage increments. Ordinate values are shown by the numerals on the graphs. The graph for 35°, 1st period, is added for comparison.

The three upper graphs illustrate how great may be the difference between cultures without artificial aeration and those with an air flow of 1 l. per day. The cultures without air flow had practically no convection and the oxygen supply to the seeds must have been very slow indeed. Perhaps the marked effect of the slowest rate of bubbling, in giving increment values higher than the corresponding ones without air flow, may have been due to stirring of the solution rather than to the entrance of air into the flasks. A double optimum is again shown on the graph for 24°, 3rd period, and it is suggested for 30°, 2nd period, the increment value for a 1-l. flow being higher than that for a 3-l. flow. For air flows of from 6 l. to 30 l. per day the 6-hr. increment values are about the same on each one of the lower three graphs, but the second 6-hr. period with 30° gave the highest increment (57 per cent.) with the most rapid air flow and might have given still higher values with still more rapid rates of flow.

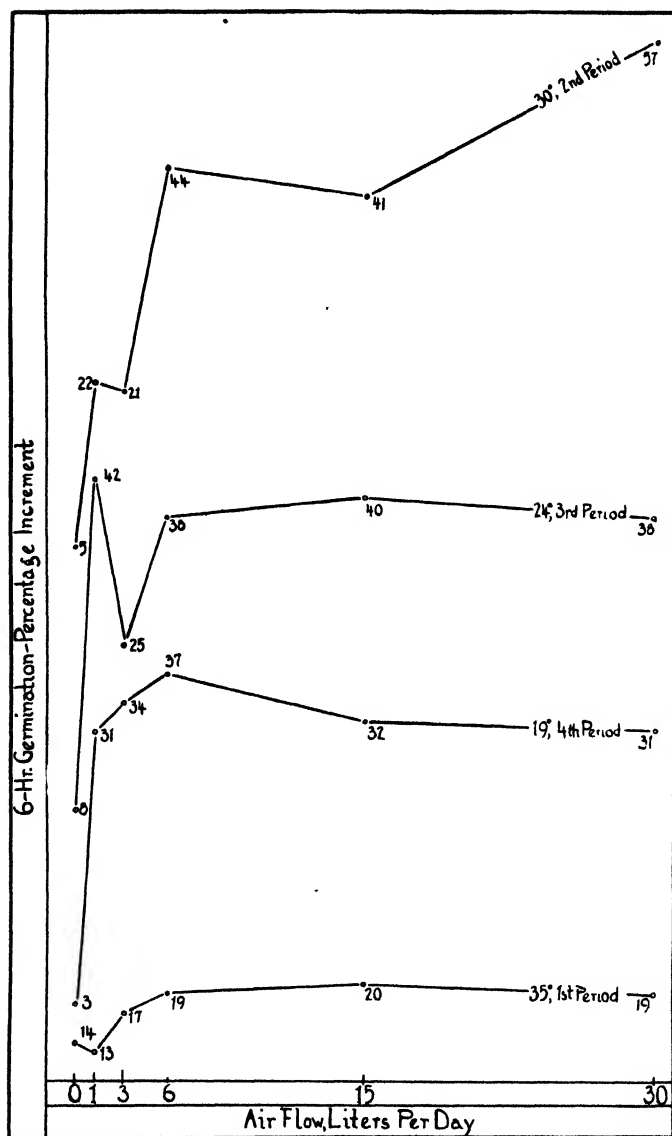


FIG. 5. Representative aeration graphs of the 6-hr. increments of germination percentage. The graphs are arranged to avoid intersections, without reference to the scale of ordinates, but all are plotted on the same scale, ordinate values being shown at the points of record.

If it were desired to select a large number of seedlings as nearly alike as possible, as for a series of solution cultures, about 50 per cent. of this lot of seed might be so selected by taking just those seedlings that had burst the seedcoats in the second 6 hours of incubation under the weak nutrient solution here used and with maintained temperature of 30° and air flow of 30 l. per day. This is an illustration of the manner in which a number of physiologically similar seedlings may be selected by means of differential culture, as has been pointed out by HAASIS. Of course this sort of selection cannot be applied without bringing the seeds into activity and the seeds selected cannot be returned to ordinary storage; they must be allowed to continue their development after it is once started, unless, indeed, they might be held in a kind of dormancy by the employment of suitably low temperature. A study of table II will suggest many ways by which several groups or categories of seedlings might be secured, the individuals of each group being nearly alike by the physiological test of germination in the same short period under a standard set of environmental conditions, while the several groups would differ among themselves in more or less pronounced ways. Seedlings produced in the first 6 hours of incubation with a specified environmental complex might be very different in physiological characteristics from those produced in the fourth 6-hr. period under the same conditions, for example. Whether such differences might be related to genetic characteristics is a question that may be interesting and important.

It will be noted from table II that, for all aeration treatments, germination at 45° was confined to the first 6-hr. period, which has already been mentioned, and that the same is practically true for 40°. With a temperature of 35° most of the germination that occurred is shown for the first 6-hr. period in every case, but the second period gave considerable germination with air flows more rapid than 1 l. per day. With this same temperature still later periods show increments that are nearly or quite negligible, excepting in the case of the most vigorous aeration. These three temperatures (45°, 40°, and 35°) are all above the general temperature optimum for this lot of seed and it appears that they agreed in giving the highest germination percentage for the first 6 hr.

Besides the relations here mentioned, other interesting relations may be noted by a study of table II. The 12-hr. increments of average germination percentage show about the same relations, *mutatis mutandis*, as do the 6-hr. increments and they need not be dwelt on here, for the 6-hr. increments are more useful in studying these relations.

OPTIMAL CONDITIONAL COMPLEXES

From what has been said in the preceding sections it is clear that there are at least three quite different criteria by which we may judge which ones

of the many different combinations of temperature, aeration treatment and incubation period were optimal.

(1) In the first place, most students of germination percentage or seed viability would probably regard as optimal, within the ranges of this study, those sets of conditions that gave the highest average percentages, without regard to time. On that basis, as has been pointed out, we may name three combinations as in the optimal range, namely: 24°, 6-l. flow, 24 hr.; 24°, 15-l. flow, 24 hr.; 24°, 30-l. flow, 24 hr. Because the average percentage values for these three combinations are all nearly 100 (being 92, 95 and 95, respectively) it is possible to say that no other combination of influences could have given percentages significantly greater. Other equally effective combinations might of course be found, especially if the period of incubation were prolonged beyond 24 hr., but these three are surely as truly optimal as any combination that might be tested. Because all three of these optimal combinations are characterized by the temperature value of 24° we have said that the general temperature optimum for nearly complete germination is 24°. And because all three are characterized by the duration value of 24 hr. we may conclude that 24° and 24 hr. are to be taken together in defining a general environmental optimum, together with a rate of air flow of 6-l. per day or more, and the background conditions employed in this study.

(2) In the second place, from another point of view, we may regard as optimal the combination of experimental variables that gave the highest mean hourly rate of seedling production. On this basis, considering the duration factor now, we find that the optimal combination, within the whole range of this study, is 30°, 30-l. flow, 12 hr. This combination gave an average hourly rate of 6.2 seedlings per hour and no other combination tested approached that value more nearly than 5.0. It is apparent that no combination of these background conditions with a maintained temperature higher than 30° might have given a higher hourly rate, nor is there any suggestion that any incubation period shorter or longer than 12 hr. might have given higher rates, unless aeration were still more vigorous than is represented by an air flow of 30 l. per day. It is logically possible, however, that some rate of air flow more rapid than 30 l. per day might have given an average hourly rate of seedling production somewhat greater than 6.2. We may therefore conclude that the optimal complex for this lot of seed, within the parameter of this study, and on the basis of the time rate of seedling production, is either the one noted above or else some combination of 30° with a 12-hr. period and an air flow more rapid than 30 l. per day.

(3) From still another viewpoint, we may regard as optimal, in a special sense, the environmental complex corresponding to that particular portion

of the first 24 hours of incubation which gave the greatest 6-hr. increment in the numerical index of seedling production. On this basis we find an optimal set of conditions defined as follows: 30°, 30-l. flow, 2nd 6-hr. period. This complex gave an increment of 57 per cent. and no other combination tested approached that value at all closely. As by the second criterion (hourly rate), the optimal temperature is here shown as 30°, the optimal rate of air flow is to be considered as 30 l. per day, or else perhaps a still higher rate.

From these and similar considerations it is clear that the optimal temperature for seedling production by this lot of seed, under the background conditions of these studies, may be stated as about 24° or about 30°, according to the criteria used. Either statement may be regarded as correct. This illustrates the principle that no concept of optimal temperature, etc., can be generally clear unless the corresponding or concomitant non-temperature influences and the criteria for judgment are specifically stated.

Special experiments

The results of the routine experiments reported in the preceding section show many combinations of temperature, aeration treatment and length of incubation period that gave average germination percentages smaller than the largest value obtained, which was 95, for 24°, 24 hr. and an air flow of 15 l. or 30 l. per day. It is suggested at once that a large proportion of these low-efficiency combinations were deficient with respect to time only, that they might have given much larger germination percentages if the tests had been continued longer. Circumstances did not permit the longer-period tests that are thus suggested but two like experiments were carried out with an incubation period of 3 days, the seven maintained temperatures regularly employed, and an air flow of 15 l. per day, other details being according to the routine specifications. The average percentage values from these two experiments are given below, along with the corresponding 24-hr.-15-l. values and also the 24-hr.-30-l. values (from table I).

	12°	19°	24°	30°	35°	40°	45°
3-day values, air flow of 15-l. per day	95	95	95	87	28	13	14
1-day values, air flow of 15-l. per day	29	76	95	84	33	22	15
1-day values, air flow of 30-l. per day	29	82	95	93	45	21	15

It is remarkable that the incubation period of 3 days gave the same germination percentage for 12°, and 24° and that this value (95) is the same as the maximum percentage obtained with the combination of 24° and 15 l. or 30 l. and 1 day. It appears that this lot of seed was capable of showing a germination percentage of about 95 with temperatures from somewhat below 12° to about 24° (or perhaps even somewhat higher), with air flow of 15 l. or more, provided the incubation period were sufficiently prolonged. It is also indicated that the combination of 24° and air flow of 15 l. gave no more germination with a longer period than with a period of 1 day; with this combination it appears that all viable seeds had germinated by the end of the 1-day period. Just how long it took to attain the percentage value of 95 with combinations of 12° and 19° with air flow of 15 l. is of course not shown, but that value was surely attained within 3 days of the beginning of soaking. For 30° we may say that the 1-day period and the 3-day period gave about the same percentage value (87, 84) with air flow of 15 l. Whether the value for 1 day and a flow of 30 l. (93) is significantly higher than this may perhaps be questioned and it is at least possible that a period somewhat longer than 3 days might have brought the percentage value for 30° and a flow of 15 l. up to 95. There is obvious experimental discrepancy between the 1-day and the 3-day values for 35° and 40°, but it is at least indicated that all of the seeds that were able to germinate at either of these temperatures had burst their seedcoats by the end of the first day under the specified conditions; for this aeration treatment and either of these temperatures the percentage value is surely no greater for the 3-day period than for the 1-day period. The data for 1 day and 3 days with a temperature of 45° are in excellent agreement; as has been emphasized, all seeds capable of germination at this supra-optimal and surely injurious temperature had apparently burst their seedcoats within the first 6 hr. of incubation, the rest dying eventually.

Cultures held for 6 hr. at 45°, with an air flow of 15 l. per day, and then transferred to 24° with the same aeration for the following 18 hr. gave the same germination percentage as was shown for the first 6 hr. at the higher temperature; about 15 per cent. of the seeds germinated in the first 6-hr. period at 45° and no additional ones germinated in the succeeding 18 hr. at 24°. This supports the conclusion that the seeds that could not germinate in 6 hr. at 45° were probably all dead by the end of that short period.

From a special series of cultures of the sort used in the routine part of this study, with the same seven maintained temperatures and with an air flow of 3 l. per day, the seeds that had burst their seedcoats in the first 6 hr., in the second 6 hr., and in the second 12 hr. were segregated and planted in 21 pots of sand, which were kept in a greenhouse room for four weeks, with daily watering. Most of these lots of barely germinated seeds produced

nearly as many apparently healthy plants as there were seeds in the respective lots, indicating that the seeds had not been injured by the culture treatment employed for germination.

About half of the lot that had burst the seedcoats in 6 hr. at 45° failed to produce plants. This seems to indicate that about half of the germinated seeds from the 6-hr.-45° culture had been seriously injured in that short period, so that they either were dead at the end of the period or died without further development after they had been planted in the pots. The plants that were produced from this lot appeared to be somewhat retarded in their growth and may not have been quite as healthy as those from germination cultures at lower temperatures. Only a very few germinated seeds were available from the cultures at 45° for the second 6 hr. and for the second 12 hr. of incubation, and no plants were obtained from either of these lots. As far as this additional evidence goes, it supports the tentative conclusion reached from the results of the routine experiments, that a maintained temperature of 45°, with the non-temperature conditions of this study, acted injuriously on most of the seeds even in the first 6 hr.

Summary

In this paper are reported results of an experimental study of the germination performance of a lot of pure-line wheat seed (Nittany variety) under different sets of environmental conditions. The study was carried out at the Laboratory of Plant Physiology of the Johns Hopkins University, under guidance of Professor Burton E. Livingston. All apparently imperfect seeds and all seeds that floated on the nutrient solution used were discarded before each experiment was begun. In each germination test 100 seeds lay on the bottom of a germination flask, under 100 ml. of nutrient solution the surface of which was about 2 cm. above the seeds. Seven temperatures (12°, 19°, 24°, 30°, 35°, 40° and 45°) were employed by means of unlighted chambers, for incubation periods of 6 hr., 12 hr., 18 hr. and 24 hr. Six different aeration treatments were employed for each combination of temperature and length of incubation period. By one treatment the cultures were without air flow or agitation, the flask being open to the air of the chamber through an ordinary 2-hole rubber stopper. By the remaining treatments air from outside the temperature chamber was continually bubbled through the solution, renewing the supply of oxygen, removing volatile excretions and insuring the stirring of the solution about the seeds. Five rates of air flow were employed: 1 l., 3 l., 6 l., 15 l. and 30 l. per day. There were consequently 42 different environmental complexes, each being tested with four periods of incubation. The medium was a 3-salt solution containing per liter 0.0084 gram-mol. of each of the salts, KH_2PO_4 , MgSO_4 , $\text{Ca}(\text{NO}_3)_2$. It had a total osmotic value of about 1

atmosphere at 20°. The culture solution was not renewed throughout the incubation period. At the end of each test the number of seeds that had germinated was ascertained and recorded as a percentage of the total number in the flask. Germination was considered as having occurred in all instances where the seedcoat had been ruptured and the white coleoptile was visible through the opening. Each test was performed five times and the five percentages were combined as the average germination percentage for that test.

The results are presented in tabular form and in representative graphs of several sorts. Almost all (95, 92 per cent.) of the seeds of a sample germinated in 24 hr. with the best environmental complexes, and high percentages (86, 83 per cent.) were obtained in 18 hr. under the best conditions. About a fifth of them germinated in 6 hr. under the best conditions.

In general, higher temperatures gave higher germination percentages, up to a temperature optimum, beyond which still higher temperatures gave lower percentages. The optimal temperature was lower as the incubation period was longer, being about 30° or 35° for the shortest period and about 24° for the longest. Aeration treatment exerted little if any influence on the optimal temperature for any period.

For 12°, 19°, 24° and 30° the germination percentages secured with any aeration treatment were progressively higher with longer incubation periods and the highest values were obtained with these temperatures and 24 hr. of incubation. On the other hand, this relation was not so clear for still higher temperatures and did not appear at all for 45°. The seeds that germinated at this highest temperature burst their seedcoats within 6 hr. and longer periods gave no higher values. Aeration treatment was without marked influence on this relation.

In general, progressively more vigorous aeration treatments gave progressively higher germination percentages, up to a rate of air flow of 3 l. or 6 l. per day, but no farther, and this relation was increasingly evident for progressively longer periods. For the longest period (24 hr.) temperatures of 19°, 30° and 35° gave progressively higher values up to the most rapid rate of air flow (30 l. per day) and it appears that still more vigorous aeration might have given higher values than were given by the 30-l. flow. With a temperature of 24° a double aeration optimum is clearly shown for the 18-hr. and 24-hr. periods and such a double optimum is suggested for 19° and 24 hr. and for 19° and 18 hr. The bimodal aeration graph representing this double optimum has a marked secondary minimum corresponding to an air flow of 3 l. per day. That is, for the instances just mentioned rates of flow of 1 l. and of 6 l. per day gave notably higher percentages than were given by the intermediate rate of 3 l. per day.

Some representative 3-dimension graphs are shown, with contours or isopleths passing through points that represent combinations of conditions giving like percentage values.

Average hourly rates of seedling production are presented and discussed, also the 6-hr. increments of average germination percentage.

As to optimal conditional-durational complexes, three complexes gave percentage values above 90, all for 24° and 24 hr., with air flow of 6 l., 15 l. and 30 l., respectively. These combinations of the experimental variables might be taken to represent general optimal conditions. If, however, we consider as optimal the combinations that gave highest average hourly rates of seedling production we must regard as optimal the combination of 30°, 12 hr. and an air flow of 30 l. per day. This gave an average hourly rate of 6.2 seedlings per day and no other combination tested gave a corresponding hourly value approaching this more nearly than 5.0. Furthermore, if we regard as optimal the complex corresponding to that particular 6-hr. interval (of the first 24 hr. of incubation) which gave the greatest 6-hr. increment in the numerical index of seedling production, we should select the second 6-hr. period with 30° and an air flow of 30 l. per day. This complex gave an hourly increment of 57 per cent. which is not at all closely approached by any other corresponding value.

Some special experiments are briefly reported. From an experiment series with an incubation period of 3 days it appears that this lot of wheat seed was capable of showing a germination percentage of about 95 with temperatures from somewhat below 12° to about 24° (or perhaps higher), with daily air flow of 15 l. or more, provided the incubation period were sufficiently prolonged. This may be true for 30° also. For 35° and for 40°, with air flow of 15 l. the 3-day cultures failed to give greater percentage values than were given by single-day cultures. For 45° no more seeds germinated in 3 days than in 6 hr.

Cultures held for 6 hr. at 45° with air flow of 15 l. per day and then subjected to 24° with the same aeration for the succeeding 18 hr., gave a germination percentage of about 15, the same as was given by the first 6 hr. of this test. This supports the conclusion that the seeds that could not germinate in 6 hr. at 45° were probably all dead by the end of that short period.

Seeds that had germinated in the first 6 hr., in the second 6 hr. and in the second 12 hr. of incubation, with air flow of 3 l. per day, and had then been planted in pots of sand and treated for four weeks like ordinary potted plants in the greenhouse, generally showed healthy and usual growth, which indicates that the germination treatment had not been injurious. But about half of the seeds that had germinated in the first 6 hr. at 45° (with 3-l. air flow) failed to continue their development in the pot cultures;

these had been killed in the 6 hr. germination period or else they had been so injured that they died shortly after being planted in the pots.

The results of this study clearly show how the germination performance of this lot of wheat seed was influenced by the experimental variables (maintained temperature, aeration treatment and duration of incubation) and how the influence exerted by any one of these variables was itself influenced by the others. They emphasize again the general principle that environmental influence on organisms needs to be studied and described in terms of environmental complexes rather than in terms of only one or a few of the influential conditions. The influence of any kind of environmental condition or "factor" can be usefully described only with adequate reference to all the other kinds of influential conditions acting at the same time.

It is pointed out that germination with a series of different but simultaneous environmental complexes and different incubation periods might be employed to secure several batches of barely germinated seeds from the same seed stock, the individuals of each batch being rather nearly alike physiologically while the several batches might be nearly alike or notably different, as might be desired. When like batches of seedlings from a given seed stock are needed for experimentation on environmental influences as they affect subsequent developmental phases, such physiological selection as is here suggested may be useful, in addition to the commonly employed selections based on general appearance, size, color, weight, specific gravity, and other morphological characteristics of resting seeds.

The relations here brought out between germination percentage, on the one hand, and the prevailing environmental complexes of the germinators and the duration of incubation, on the other hand, may be useful also in the further working out of standard procedures for ordinary seed testing.

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DISCHARGE OF SACCHARASE FROM MYCELIUM OF *PENICILLIUM GLAUCUM*

ZOLTAN I. KERTESZ

(WITH TWO FIGURES AND ONE PLATE)

Introduction

The relation between the composition of the media of fungus cultures and the enzymes produced has been the subject of many investigations. There is very little known about the discharge of the enzymes secreted by the mold into the culture medium. This phenomenon bears the most intimate relationship to the rôle played by the enzymes and to the life-cycle of the molds and it therefore merits more attention.

The first recorded data in the literature that deal with the diffusion of mold enzymes into the medium are found in the study of FERNBACH (7) on the saccharase of *Aspergillus niger*. His method can not be regarded as free from objections, (5) but he established the first enzyme unit and he was the first to point out the great importance of the degree of acidity of the reaction mixtures. He measured the distribution of the saccharase between the mold and medium. The main result of his investigations was the discovery of the fact that the total amount of saccharase (activity) expressed in his enzyme unit was fairly constant, decreasing only about 20 per cent. from the second to the fifth day. The distribution ratio of the enzyme between the mold and medium changed during growth. On the second day 3 per cent., and on the fifth day 37 per cent. of the total enzyme content was found in the medium.

The conclusion to be derived from FERNBACH's experiment is that the maximum amount of enzyme is formed during the first two days, and later this enzyme simply passes slowly from the mold to the medium. He obtained practically the same results with a yeast,—*Saccharomyces pastorianus*. Since FERNBACH's experiments no work has been done on the distribution of the enzyme between microorganism and medium.

In the experiments of DOBY and KERTESZ (2) the saccharase content of *Penicillium glaucum* cultures was determined and the enzyme content of the medium was found to be very low. These authors were studying the changes in the saccharase content of the mold when grown with and without potassium, and therefore did not pay further attention to the enzyme content of the medium.

BRIDEL and AAGAARD (1) have shown that when the mold is kept on distilled water, the enzyme diffuses out of the mycelium into the water.

IWANOFF and KUDRJAWZEWA (8) published a paper in 1929 entitled,

“Ausscheidung der Saccharase aus der Zellen.” The main conclusions drawn by these authors were that the discharge of the saccharase from the mycelium depends on the pH of the medium; very little saccharase was discharged if the medium was acid but as it became more alkaline more and more enzyme was discharged into the medium. In this connection it should be pointed out that one essential weakness of the method used by the Russian authors is that the saccharase content of the medium only has been studied and never that of the mold. For this reason the data that these workers presented can not be considered sufficient to show completely the extent of the discharge of the saccharase. Although the enzyme content (capacity) of the medium of two different cultures may be found to be the same, this part of the enzyme content may be only 10 per cent. in one case but 90 per cent. of the total enzyme content produced by the mold in the other case.

It was for the purpose of securing more information concerning the factors influencing the discharge (diffusion) of saccharase from the cells of *Penicillium glaucum* that the studies reported in this paper were undertaken.

Experimental

METHOD

The strain of *Penicillium glaucum* Link used in these experiments was the same as used in earlier experiments, the culture originating from the Royal Hungarian Institution for Industrial Fermentations in Budapest, Hungary.

The salt supplement of the cultures was always the same as given in earlier papers (10, 11, 2) and contained sufficient amounts of P, K, Mg, Ca, Na, Cl, SO₄, N, (NO₃ and H₄ N) and traces of Fe⁺⁺ and Zn. After inoculation the mold has been grown in 100-cc. Erlenmeyer flasks containing 50 cc. of medium and at a temperature of 24.0° C.

The procedure used in the enzyme determination was as follows: The mold was taken off of the medium and washed with a quantity of distilled water such that the volume of the medium was restored to 50 cc. as at the beginning of the growth. The dry matter was determined on a small sample of mycelium, drying to constant weight at 95° C. The rest of the mold was ground in a porcelain mortar and suspended in water. A sample of this suspension was taken for the determination of the dry matter content of the suspension and the remainder was used for the determination of the enzyme activity.

The medium was filtered before being used for the determination of the enzyme activity. The reaction mixtures contained in all cases 5 per cent. of sucrose. The amount of sucrose contained in the part of the medium

used has always been determined and correction for this has been made in the calculations. The pH of the reaction mixture was always 4.5 which has been shown to be the optimum for this enzyme (2). The pH was determined by the use of indicators. Phosphate buffer was used throughout and 3 per cent. of toluol was always added. The reaction temperature was 38.0° C. in all experiments.

At the beginning of the experiments and at the indicated intervals, samples were taken from the reaction mixtures, clarified by neutral lead acetate and sodium carbonate, centrifuged, and in the clear solution the reducing sugars were determined. In one series the optical rotation was determined in a 200 mm. tube. For the determination of the reducing sugars Bertrand's method was used and for the calculation reference was made to the author's recalculated tables (12). The values reported in the tables have been corrected for the blank found at the beginning of the experiment.

The monomolecular reaction constants were calculated by the use of the familiar formulae:

$$k = \frac{1}{t \times 0.4343} \cdot \log \frac{A}{A - X} \quad (1)$$

$$\text{and} \quad k = \frac{1}{t \times 0.4343} \cdot \log \frac{\alpha_0 - \alpha_\infty}{\alpha - \alpha_\infty} \quad (2)$$

in which "t" is reaction time (min.), A is the amount of substrate (sucrose) present at the beginning of the reaction, X is the amount of substrate changed in time "t," α_0 is rotation at the beginning, α is rotation after the time "t." The value of α_∞ is calculated from the formula

$$\alpha_\infty = \alpha_0 (0.417 - 0.05 t). \quad (3)$$

The If is calculated from v. EULER's formula (6)

$$\text{If} = \frac{k \times \text{gm. sucrose in the reaction mixture}}{\text{gm. of dry matter of the enzyme preparation in the reaction mixture}} \quad (4)$$

In the case of the enzyme determinations in the medium "cc. of medium used in the reaction mixture" has been substituted for "gm. dry matter."

The enzyme content of the whole culture of the mold and medium respectively has been expressed in the formula proposed by the author (13):

$$\text{Total enzyme content of the mold} = E_1 = (If_1) \times (\text{dry matter yield in gm.}) \quad (5)$$

$$\text{Total enzyme content of the medium} = E_2 = (If_2) \times (\text{total volume of the medium in cc.}) \quad (6)$$

Before presenting the data obtained, it is necessary to explain why the above method of determination has been used in preference to the method described by IWANOFF and KUDRJAWZEWA. As mentioned before, the Russian authors determined the enzyme content in the medium only. Further, the method used for their determinations was not free from objections. Their method as described in their paper was as follows (*loc.*

cit., p. 243): "Von 50 ccm. Kulturflüssigkeit wurden 10 ccm. Filtrat entnommen und 10 ccm. Wasser und 1 gm. Saccharose hinzugefügt; das Medium wurde auf pH = 5.2, $t = 52^{\circ}$ C. gebracht; nach Verlauf einer bestimmten Zeit, 1 bis 3 Stunden, wurde die Menge des reduzierenden Zuckers nach Bertrand bestimmt. Die Saccharasekraft wurde in Milligrammen reduzierenden Zuckers ausgedrückt, welcher sich aus 1 gm. Saccharose gebildet hat. Diese einfache Methode wurde bei allen weiter beschriebenen angewandt."

It is to be seen that no determinations of the reducing power were made at the beginning of the reactions. The media used by IWANOFF and KUDRJAWZEWA contained sugars in most cases and perhaps other substances which have a reducing power; therefore their single determination of the reducing power could scarcely be expected to define the rate of reaction. If the mold was grown, for instance, on sucrose solution the original reducing power of the medium was changed considerably by the inversion of the sucrose. These two opposing reactions (1) the inversion of the original sucrose, and (2) the disappearance of the invert sugar from the solution, make the results obtained by the Russian authors so uncertain, that from their work no more than qualitative conclusions can be drawn.

RESULTS

On the basis of the experiments of IWANOFF and KUDRJAWZEWA attention was first paid to the influence of the reaction of the medium on the discharge of the saccharase to the medium.

In these experiments the *Penicillium glaucum* was grown on 50 cc. of medium containing 5 per cent. sucrose and inorganic salts. By the use of N/10 NaOH or N/10 H_2SO_4 the reaction of the medium was brought to the desired pH. A few drops of solutions of suitable indicators were added and the pH corrected daily throughout the experiment by the addition of base from a sterile burette. Of course, during one day the production of acid by the living mold caused a certain shifting toward the acid reaction, therefore the pH values given are approximately 0.5 pH higher than the lowest value actually reached during growth. The cultures were harvested on the fourth day after inoculation. In this single case the determination of the saccharase effect in the mold was done by the polarimetric method.

The results obtained show that contrary to the conclusions stated by IWANOFF and KUDRJAWZEWA, saccharase has been found in the media of cultures grown in acid reaction. On the whole the total enzyme content ($E_1 + E_2$) decreased with increasing pH. The pH of the first culture was 3.0 at the beginning, and it was necessary during growth to correct it, because it had decreased to 2.7–2.8. From this experiment it is to be seen that the saccharase of *Penicillium glaucum* passes into the medium in quite acid reaction.

TABLE I
SACCHARASE CONTENT OF THE MYCELIUM AND MEDIUM OF *Penicillium glaucum* CULTURES, GROWN AT DIFFERENT
PH'S ON 5 PER CENT. SUCROSE FOR 4 DAYS

PH OF THE MEDIA	DRY MATTER YIELD	SACCHARASE IN THE MOLD			SACCHARASE IN THE MEDIUM		$(E_1 + E_2) \times 10^3$	TOTAL SACCHARASE IN THE MEDIUM
		DRY MATTER IN THE DETERM- INATION	AVERAGE $k \times 10^4$	$If \times 10^3$	$E_1 \times 10^3$	AVERAGE $k \times 10^4$	$E_2 \times 10^3$	
3.0	gm. 0.115	gm. 0.1319	10.61	24.13	3.74	0.37	0.26	per cent. 6.5
4.1	0.111	0.0968	9.19	27.57	3.06	0.59	0.41	11.8
5.1	0.067	0.0549	4.03	22.02	1.48	0.51	0.36	19.6
6.3	0.043	0.0383	6.79	53.20	2.28	1.46	1.02	30.9
7.0	0.026	0.0214	2.11	29.57	0.77	0.45	0.31	28.7
7.9	0.028	0.0236	2.83	35.94	1.01	0.23	0.16	13.7
8.3	0.025	Not determined				0.40	0.24	(0.24)
9.8	Fragments of mycelium					0.26	0.18	(0.18)

TABLE II
SACCHARASE CONTENT OF THE MYCELIUM AND MEDIUM OF *Penicillium glaucum* GROWN ON 50 CC. OF 5 PER CENT. SUCROSE SOLUTION AND INORGANIC SALTS

[illegible]

The rate of growth, as is to be seen from the dry matter yield, varied materially in the cultures grown at different pH's. All the cultures were therefore in different stages of development, and presumably some of them near to and some far from having their highest saccharase content. DOBY and KERTESZ found that the mycelium of *Penicillium glaucum* grown on 5 per cent. sucrose had the highest saccharase content on the fifth day of growth. This work showed the effect of pH on the growth of the mold, but no attempt was made to arrive at conclusions in regard to the distribution of the enzyme between mycelium and medium. For this reason the changes of the saccharase content of growing cultures were studied as will be shown later in this paper.

In the following three experimental series given in tables II, III and IV the mold was grown on 50 cc. of a medium which contained the inorganic salt supplement and 5 per cent. of sucrose (II), the salt supplement and 5 per cent. of sucrose and 0.25 per cent. of asparagin (III), and the salt supplement and 1.5 per cent. solution of Witte peptone (IV).

As can be seen from table II, the dry matter yield increased even after the eleventh day, showing that the supply of nutrients had not been used up during that time. The enzyme content of both mycelium and medium was the highest on the fourth day. The highest enzyme content of the medium was observed on the fourth day and amounted to 17.7 per cent. of the total enzyme content.

As was seen from the pH values of the growing cultures, the reaction of the medium was shifted toward the acid side very quickly, i.e., the original reaction of pH 5.4 was changed to 2.9.

The results presented in table III are similar to those presented in table II. The growth is more rapid, and because of the larger amount of nitrogenous and carbohydrate nutrients the dry matter yield is higher also. The total enzyme (or enzyme activity) produced by the culture at the fourth day is double that of the culture having no asparagin. The rate of increase of the actual acidity in the medium is a little slower in the presence of asparagin, possibly because of the buffer action of this compound.

The maximum enzyme activity per unit of dry matter (If_1) is only 10 per cent. higher than in the culture without asparagin, but the enzyme content of the medium is much higher. But even in this case only on the fourth day was 25 per cent. of the total enzyme content found in the medium; later this value decreased to 5 per cent., only one twentieth of the total enzyme contained in the whole culture.

The results obtained with cultures grown on Witte peptone are presented in table IV. In no case has a definite saccharase activity been found.

TABLE III

SACCHARASE CONTENT OF THE MYCELIUM AND MEDIUM OF *Penicillium glaucum* GROWN ON 50 CC. OF 5 PER CENT. SUCROSE, 0.25 PER CENT. ASPARAGIN, AND INORGANIC SALTS

AGE	ACIDITY OF THE MEDIUM	SACCHARASE IN THE MOLD					SACCHARASE IN THE MEDIUM							
		DRY MATTER IN THE DETERMI- NATION	IN- CREASE IN INVERT SUGAR IN 6 HOURS	$k \times 10^4$	14×10^3	DRY MATTER YIELD	$E_1 \times 10^3$	USED FOR THE DE- TERMI- NATION	IN- CREASE IN INVERT SUGAR IN 6 HOURS	$k \times 10^4$	SUCROSE IN THE MEDIUM	$E_2 \times 10^3$	$(E_1 + E_2) \times 10^3$	TOTAL SAC- CHARASE IN THE MEDIUM
days	pH	gm.	mg.			gm.		cc.	mg.		per cent.			per cent.
2	4.4	0.1010	15.0	4.29	12.74	0.141	1.33	30	0.3	0.06	3.41	0.04	1.37	3.3
4	2.9	0.2950	75.4	41.62	42.40	0.703	29.3	30	51.3	18.64	0.44	9.73	39.03	24.7
7	2.7	0.9048	36.9	11.71	38.91	1.279	4.97	30	17.4	5.04	0.29	0.26	5.23	5.0
11	2.7	0.3958	21.4	6.34	4.80	1.268	6.08	35	24.0	7.22	0.44	0.32	6.40	5.0
16	2.7	0.3010	15.6	4.47	4.46	1.498	6.68	30	26.5	8.08	0.32	0.42	7.10	5.9

TABLE IV
SACCHARASE CONTENT OF THE MYCELIUM AND MEDIUM OF *Penicillium glaucum* GROWN ON WHITE PEPTONE AND INORGANIC SALTS

Age	Acidity of medium	SACCHARASE IN THE MOLD										SACCHARASE IN THE MEDIUM										PRESENCE OF ENZYME DETERMINED BY THE									
		DETERMINATION AFTER HOURS										DETERMINATION AFTER HOURS																			
		DRY MATTER IN EXPERIMENT		0		6		24		0		6		24		Used for the determination		Rotation		Invert sugar		Rotation		Invert sugar		Reducing method					
		Rotation	Invert sugar	Rotation	Invert sugar	Rotation	Invert sugar	Rotation	Invert sugar	Rotation	Invert sugar	Rotation	Invert sugar	Rotation	Invert sugar	Rotation	Invert sugar	Rotation	Invert sugar	Rotation	Invert sugar	Mold	Medium	Mold	Medium						
days	pH	gm.	deg.	mg.	deg.	mg.	deg.	mg.	cc.	deg.	mg.	deg.	mg.	deg.	mg.	deg.	mg.	deg.	mg.	deg.	mg.	deg.	mg.	deg.	mg.	(+)	-	-	-	+	+
2	6.9								30	0.398	3.3	0.404									0.422	2.0				(+)	-	-	-	+	
4	7.4	0.037	0.552	2.6	0.514	1.1			30	0.339	3.3	0.474									0.458	1.7				(+)	-	-	-	+	
7	7.6	0.084	0.474	2.9	0.425	3.4			30	0.452	2.6	0.434	2.6								0.458	1.8				-	-	-	-	+	
11	7.4	0.062	0.524	1.9	0.550	3.0			30	0.418	2.0	0.396									0.402	4.0				-	-	-	-	+	

It is to be seen from the data presented that the question of the diffusion of saccharase to the medium in mold cultures is much more complicated than it has been supposed by earlier authors. With changes in the medium, changes occur in the whole life-cycle of the mold. On account of the different substrates, the molds produce different amounts (activity) of enzyme. Furthermore the enzyme content changes with the age of the culture.

In the following experiment mold grown on 5 per cent. sucrose for five days was kept immersed in buffer solutions of various pH in the presence and also in the absence of toluol and the enzyme diffused to the buffer solutions after two days was determined. The mold had been grown in five 100-cc. Erlenmeyer flasks each containing 50 cc. of medium. The weight of mycelium harvested on the fifth day was 13.75 gm. The dry matter content was determined to be 19.15 per cent. The total dry matter yield of the five cultures was therefore 2.63 gm. After washing and drying with filter paper it was divided into 1-gm. portions each representing 0.19 gm. dry matter. Ten of these portions were put in small bottles having glass stoppers and containing 40 cc. of a mixture of 4 cc. N/10 phosphate buffer and 36 cc. of sterilized distilled water making a final buffer solution N/100 with respect to phosphate. The mold was immersed in the solutions. At the beginning of the experiment the enzyme content (activity) of the mold was determined to be $I_f = 31.6 \times 10^{-3}$. For the determination of the enzyme diffused to the solutions, after filtration 25 cc. was mixed with 20 cc. of a 12.5 per cent. sucrose solution and 5 cc. of phosphate buffer, pH = 4.5. In several cases the solutions were too far removed from pH 4.5 to use directly and in these the pH was corrected by the addition of N/20 NaOH or N/20 H_2SO_4 . From these reaction mixtures 10 cc. was taken out at the intervals indicated, clarified by neutral lead acetate and sodium carbonate, and made up to 100 cc. This solution was used for the determination of the reducing sugars produced by the action of the saccharase. In figure 1 are shown the results of these experiments.

In all these last experiments containing buffer solutions the enzyme activity was higher than in the blank in which the mold had been kept on sterile distilled water. The shape of the curves representing the relations between pH of the solutions in which the mold was kept and the enzyme found in it after two days are very remarkable. In the presence of toluol the highest enzyme content was found in the neutral solution, while in the absence of toluol the lowest enzyme content was found at the same reaction. The highest enzyme content recorded was found in the solution without toluol at pH 8.9. In this case the solution contained 2.8 times as much enzyme as the blank on distilled water.

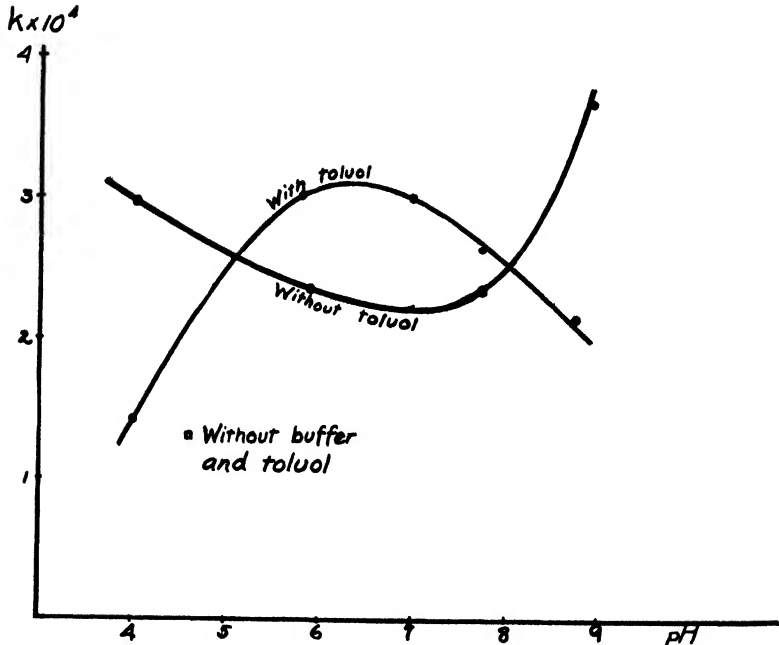


FIG. 1. The discharge of saccharase from the mycelium of *Penicillium glaucum* cultures. (The k 's are averages of the values obtained in 3, 6 and 24 hours.)

Discussion

A great deal of work has been done on the permeability of the plant cells, and on the factors influencing the diffusion of different plant materials, but among the many theories presented no one theory can be found to explain this phenomenon satisfactorily. It has been supposed that in the diffusion of different compounds through the cell wall, the VAN'T HOFF rule is not applicable. It was supposed furthermore, that besides the osmotic permeability there exists "selective permeability" (15), which would permit the diffusion of compounds like sugars and some amino-acids. It has been observed that toxic materials of presumably high molecular weight are put out from cells which contain concentrated solutions of compounds of low molecular weight, but which do not pass out of the cell at all. The passage of enzymes through cell walls is no doubt a complicated matter. A few papers dealing with the diffusion of enzymes through collodion and similar membranes have been published but very little has been done on the diffusion through cell walls.

In an earlier paper the author pointed out that the formation of enzymes bore a most intimate relationship with the necessity of the organism for them (10). In the case of a sucrose medium the saccharase was presumably

produced by the mold for the purpose of splitting the sucrose into simpler compounds which possibly could be more easily utilized by the mold than sucrose. It is entirely possible that the mold is not able to use sucrose at all, since in the case of a sucrose medium saccharase is produced quite generally. If the saccharase is produced by the cell for the purpose of splitting sucrose it is easy to understand that the amount of enzyme required should decrease after most of the sucrose of the medium has been converted into invert sugar (11).

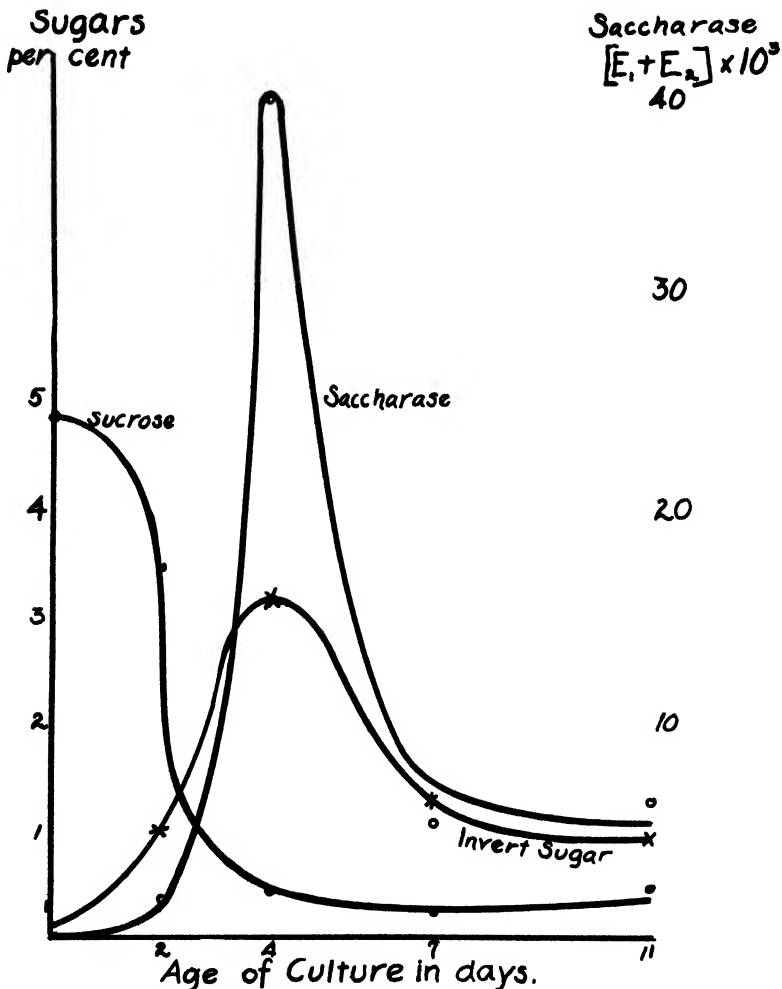


FIG. 2. The relation between sucrose and saccharase content of growing *Penicillium glaucum* cultures. (Grown on 50 cc. of 5 per cent. sucrose solution, containing 0.25 per cent. asparagin and inorganic salts.)

It is to be seen from fig. 2 that the greater part of the sucrose of the medium was inverted in the interval between the second and fourth day. In fact 63 per cent. of the total sucrose content of the medium had disappeared during this period. It is also to be seen that the saccharase content of the cultures was the highest at the fourth day of growth. After the greater part of the sucrose was converted into invert sugar the saccharase content decreased abruptly. It is interesting to note that a small amount of sucrose (or another polysaccharide?) is present even after sixteen days. A very low saccharase activity is to be found at this same time.

The results of the present experiments are not in harmony with those of the earlier investigators. Of course, the subject of their experiments has not been *Penicillium* but *Aspergillus niger*, but the author is convinced that the difference is caused more by the method of investigation than by the organism used.

The pH of the media in the experimental series in table II has been determined to be 2.9 on the second day. The discharge of the enzyme however does not start until later, since on the second day no enzyme was found in the medium. This fact is in very good harmony with the results presented in table I, where with the cultures grown on acid media, saccharase has been found in the nutrient solution. IWANOFF and KUDRJAWZEWA (p. 246) state that if cultures have been grown on a medium containing sucrose for four days, and to the medium oxalic acid is then added, no saccharase effect could be found after two or eight days.

The writer, in an attempt to show this directly, tried to grow cultures (*Penicillium* and *Aspergillus niger*) in the presence of oxalic acid in the amounts used by the Russian authors but no growth could be obtained. The reaction of these solutions has been determined to be around pH 1.25–1.70. Whatever saccharase is discharged to such a solution would lose its activity entirely in a very short time, because this high acid reaction has been shown to be very toxic to saccharase (4). NELSON and PALMER (14) observed that yeast saccharase is affected by a reaction of pH 4.6. Furthermore, oxalic acid itself is known to be one of the most toxic of organic acids for molds (9). With these observations of others in mind, it is obviously impossible to say from the experiments of IWANOFF and KUDRJAWZEWA that the acidity alone of the medium used has prevented the saccharase from passing out of the mycelium into the medium. Even if any enzyme should pass out, it would shortly become inactivated due to the high acidity, aggravated in this case by the toxic oxalate ion.

In the experiments with growing cultures presented in this paper the reaction of the medium always turned acid with the exception of the experiments with the peptone medium, where no definite saccharase effect could be obtained. It is to be seen from the first series, that the medium

of cultures grown at a pH of 6.3, 7.0 and 7.9 contained as much as 30 per cent. of the total saccharase content of the whole culture. The question whether this saccharase is discharged by the normal diffusion of uninjured cells or whether it is coming from the cells killed by the alkaline medium can not be decided from these experiments.

To be able to make a study of the discharge at different pH reactions, without being disturbed by the different rates of growth of the mold, an experimental series has been carried out, the results of which are presented graphically in fig. 1. The results obtained are no doubt due to the operation of a great many factors. The different parts of the curves should be explained in quite different ways, since the influence of the acid and alkaline medium is not identical.

From the first experimental series as well as from these later experiments it can be seen that the mold without toluol discharges a great deal of its enzyme content to an alkaline medium. This observation is in harmony with IWANOFF and KUDRJAWEZA'S results, who found that *Aspergillus niger* always put out more enzyme on an alkaline than on a neutral medium. On the acid side of this curve enzyme activity could be observed also. This is no doubt the result of a certain growth continued by the mold on the buffer solution. At the neutral reaction, a lower enzyme activity has been found. This reaction is certainly not favorable for the growth of the mold neither does it cause an increase of the enzyme by discharge nor by the plasmolysis of the cells.

Quite different are the results of the experiments in the presence of toluol. If the mycelium of the mold is placed into buffer solutions on which there is some toluol, the toluol is in much more intimate contact with the mold than with a suspension of the mycelium in the autolytic experiments of DOBY and KERTESZ. VON EULER and B. VON EULER-AF UGGLAS (3) established the fact, that the action of different protoplasmic poisons is quite different on living and non-living cells. They found that toluol killed the saccharase of *Monilia*, if living cells were exposed to it, but had only a very little effect, if dead (dried) cells were treated. The experiments here described show further that toluol is more toxic to the saccharase at either acid or alkaline reaction than at neutral reaction.

To make a further study of the effect of different treatments on the cells of *Penicillium*, microscopic sections have been prepared.¹ Photographs (x 80) of some of these are presented on the accompanying plate VIII. No. I is taken from a section prepared from the mold which had stood on an acid buffer solution for two days without toluol. It can be seen that there is a secondary growth quite distinct from the mycelium which had been

¹ The writer's sincere thanks are due to Dr. MABEL NEBEL and to Dr. BERNARD R. NEBEL for preparing these sections and photomicrographs.

formed on the original medium. There is also a rich spore formation to be seen. No. II is prepared from the mold from the alkaline solution without toluol. The mycelium and the layer of spores are wrinkled and the hyphae are partly empty as a result of the effect of the alkalinity. In this sample the greatest discharge of the saccharase had been found. Plate VIII no. III is taken from the section prepared from the mold from acid solution with toluol. The structure in general is very badly affected by the toluol. No secondary growth can be seen and no plasma can be observed in the sporangia. The mold in alkaline solution and in the presence of toluol is even more drastically affected (no. IV).

No doubt the present state of our knowledge is not sufficient to draw general conclusions in regard to the discharge or diffusion of enzymes. This can be done only after collecting more data and making further studies of this interesting as well as important phenomenon.

Summary

1. The discharge of saccharase in growing cultures of *Penicillium glaucum* has been studied. The highest enzyme content of the mycelium and medium has been found to occur on the fourth day of growth. Never more than one third of the total enzyme content of the culture has been found in the medium.

2. The saccharase can pass into the culture medium even at acid reaction, but the rate of discharge is lower in an acid than in an alkaline medium.

3. A marked decrease of the enzyme content of both mold and medium has been observed after most of the sucrose present in the medium was inverted.

4. When the mycelium of five-day-old mold containing saccharase ($I_f = 31.6 \times 10^{-3}$) had been kept on a series of buffer solutions of pH's 4.0–8.7, saccharase was detected in solution after two days in all cases.

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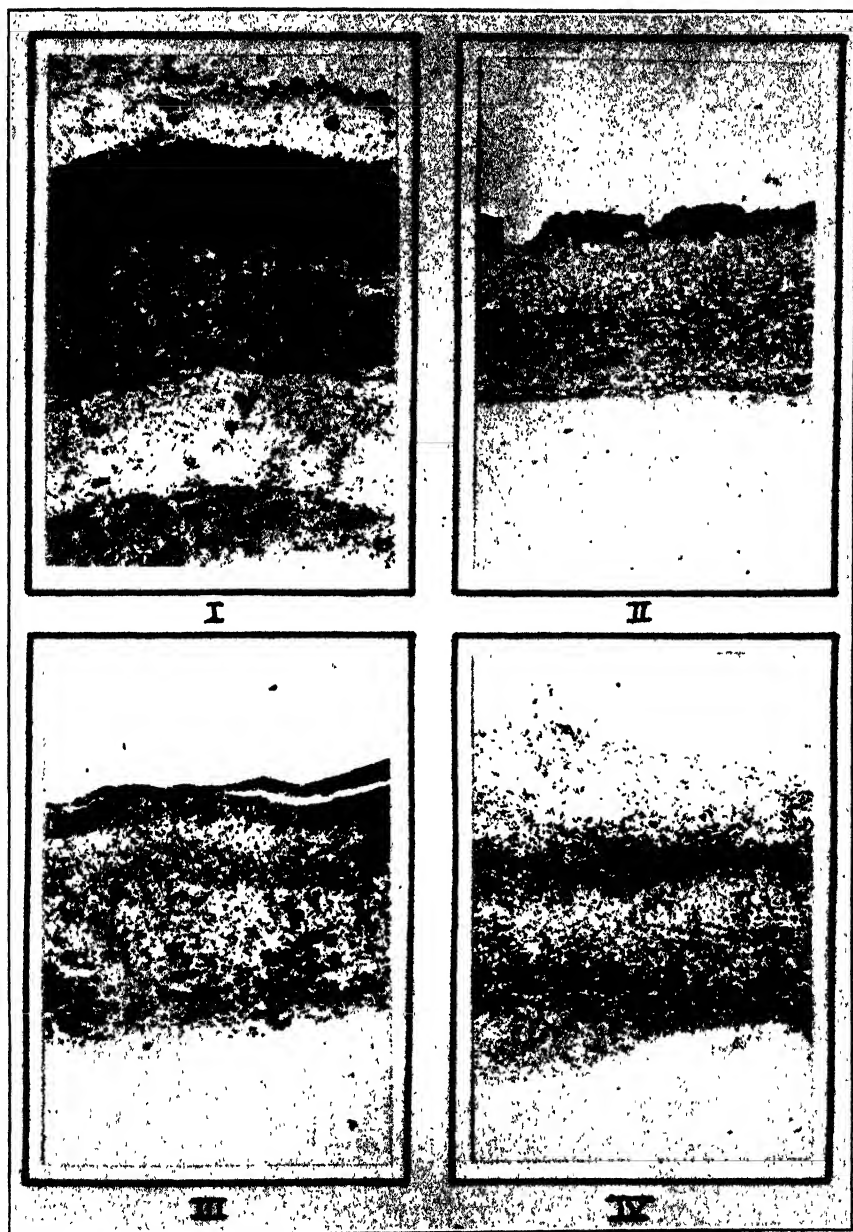
EXPLANATION OF PLATE VIII

No. I. Section from five days old *Penicillium glaucum*, which stood in acid buffer solution (pH = 4.1) for two days.

No. II. Section from five days old *Penicillium glaucum* which stood in alkaline buffer solution (pH = 8.9) for two days.

No. III. Section from five days old *Penicillium glaucum* which stood in acid buffer solution (pH = 4.0) in the presence of toluol for two days.

No. IV. Section from five days old *Penicillium glaucum* which stood in alkaline buffer solution (pH = 8.7) in the presence of toluol for two days.



KERTESZ—DISCHARGE OF SACCHARASE

LIGHT AND THE CAROTINOID CONTENT OF CERTAIN FRUITS AND VEGETABLES

LAURA LEE W. SMITH AND ORA SMITH*

Introduction

While studying the relationship of vitamin A and carotinoid content of fruits ripened under controlled conditions, it became evident that a consideration of this problem would not be complete without a study of the light factors involved. The object of this investigation was to find out, if possible, the effect of the exclusion of light from the developing fruits of various fruits and vegetables on their carotinoid content. That portion of the study of the relation of vitamin A to the carotinoid content will be presented elsewhere in a separate paper.

A search of the literature failed to disclose any work done on the effects of complete exclusion of light from the fruits of plants on their carotinoid content.

OVERHOLSER (12), interested primarily in anthocyanin formation, excluded light from the fruits of apples, pears, peaches, apricots and nectarines by means of black sacks and found that, in most varieties, no red color developed. The fruits were bagged, however, after chlorophyll had developed in them.

Material and methods

The following kinds and varieties of fruits were selected for their differences in flesh and skin color and in pigment content: Elberta and Mayflower peaches, Humboldt and Stanwick nectarines, Royal apricots, Clark's Albino, Ruby Gold, Globe, California Earliana and Gigante Ingrenoli varieties of tomato.

PREPARATION OF TREE FRUIT SAMPLES

The Elberta is a deep yellow-fleshed peach; the Mayflower an early white-fleshed peach; the Humboldt a yellow-fleshed nectarine; the Stanwick a white-fleshed nectarine and the Royal a deep yellow-orange-fleshed apricot. These fruits were selected from the station orchards at University Farm, Davis, California.

On March 31, 1928, the flowers and fruit were bagged with manila bags lined with either a heavy sized black paper (skytogen) or a double thickness of high grade thin black paper. The apricots and Elberta peaches

* These investigations were conducted at the University of California while the senior author was a member of the Household Science Department and the junior author connected with the Division of Truck Crops.

had set at the time of bagging, the former being about one centimeter in diameter and the latter about 4 millimeters in diameter; the petals of the nectarines and white peaches were beginning to fall. The bags were tied on over clusters of fruit and blossoms and not removed until harvested. The loss from drop was quite heavy with the Elberta peach and the Stanwick nectarine, and it was necessary to bag fruit again during the early part of May. Bagged fruits and a similar quantity of unbagged fruits from the same location on the tree were harvested at the same time.

The fruits were peeled, seeded, cut into small pieces and placed in small air-tight non-actinic glass bottles with glass inlet and outlet tubes. These bottles were then evacuated, the vacuum released with oxygen-free carbon dioxide and stored in the dark at -10° F.

PREPARATION OF TOMATOES

JONES and ROSA (7) state that the presence and distribution of pigments varies in different varieties of ripe tomatoes as follows: In lemon-yellow fruit carotin occurs in the pericarp, but the epidermis is colorless; in orange-colored fruit, carotin occurs both in the pericarp and epidermis; in pink fruit, lycopersicin occurs in the pericarp but the epidermis is colorless; in the red fruit lycopersicin occurs in the pericarp and carotin in the epidermis and probably in the pericarp as well. Albino or white fruits lack all pigments.

The varieties of tomatoes grown were Clark's Albino, having medium sized fruit with pale yellow flesh and colorless skin; Ruby Gold, large fruit, deep yellow flesh with the locules outlined by deep red flesh, colorless skin; Globe, pink fruit with red flesh and pale yellow skin; California Earliana, medium red flesh and orange-yellow skin and Gigante Ingrenoli having small fruit of very deep red flesh and orange-yellow skin. The plants were grown at Berkeley, California, handled in a commercial manner and pruned to one stem.

The flowers were hand pollinated and bagged immediately in the same manner as the tree fruits were handled. The fruit samples also were prepared and stored in the same manner as the tree fruits.

DETERMINATION OF PIGMENTS

As only relatively small quantities of fruits were available for analysis, a method of pigment extraction from wet samples was devised. Samples varying from 10 to 100 grams were analyzed.

Preliminary extraction experiments with the SCHERTZ (13, 14) modification of WILLSTÄTTER's method for carotin and xanthophyll, the WILLSTÄTTER and ESCHER (19) method for lycopersicin and the LUBIMENKO (9)

alcohol extraction method, were unsatisfactory because of the large quantity of fruit needed, and the loss in pigments due to the many extractions and drying. At the suggestion of Dr. J. H. C. SMITH, of the Coastal Laboratories of the Carnegie Institution of Washington, who had found pyridine (b. p. 112–115° C.) a good solvent for carotin, a method was devised using this solvent. It was possible by this method to use minimum composite samples of 10 grams. Extraction with pyridine should be performed under a well ventilated hood because of the poisonous fumes. Since there was no known method of separating lycopersicin and carotin quantitatively, except by fractional crystallization, these isomers were necessarily determined together as the petroleum ether soluble pigments. Xanthophyll was determined by treating the petroleum ether solution with 80 per cent. methyl alcohol. This pigment was present only in the green fruits in concentrations great enough to give any perceptible coloration. The method of SCHERTZ (15) for the extraction and separation of chlorophyll, carotin and xanthophyll, was used to obtain the carotinoids from the green fruit. The depth of color in the petroleum ether solution of the carotinoids was determined with a Bausch and Lomb colorimeter of the Duboscq type.

The description of the new method used for carotin and lycopersicin extraction by the use of pyridine is given in detail.

A weighed sample of the material was triturated with washed and ignited sand and transferred to an Erlenmeyer flask. Pyridine (b. p. 112–115° C.) was added in 50-cc. portions, the first portion allowed to remain in contact with the material for 30 minutes, then decanted to a separatory funnel through a glass wool filter, retaining as much of the pulp as possible in the flask. Pyridine in amounts equal to about 15 times the weight of the sample was sufficient to remove all of the pigment. To the pyridine extractions in the separatory funnel were added equal volumes of petroleum ether (b. p. 40–60° C.) and acidulated water (equivalent to approximately normal sulphuric acid). The solution should stand from 5 to 40 minutes for complete separation of the two layers. Pyridine is miscible with water in all proportions and also forms a salt with the acid which facilitates its removal. About five washings with the acid are necessary to remove the pyridine completely. The petroleum ether layer then containing the carotinoid pigments was washed with distilled water to remove all the acid, or to the point when the liquid showed no further reaction to litmus paper. The petroleum ether solution was then filtered through a layer of anhydrous sodium sulphate and made up to a volume which produced a color nearly the same as that of the standard. Glass stoppered graduate cylinders were used for this purpose.

WILLSTÄTTER's 0.2 per cent. potassium dichromate solution equal to 0.0268 per cent. carotin, and the carotin color standard of SPRAGUE (16) were adopted as the carotin color standards. The use of both these standards was found necessary, the latter matching the color of the petroleum ether solutions obtained from the tree fruits and the former matching the petroleum ether solutions obtained from the tomatoes.

The pyridine removed the lycopersicin from the tomatoes along with the carotin and xanthophyll, giving a petroleum ether solution which presumably contained all the pigments. It was observed that during these extractions the pyridine removed the yellow pigments first; on further addition of pyridine the red pigment was removed, giving a deep orange-red color to the extracts. No further attempt was made to identify the pigments in the first and the last extractions by pyridine, but this appeared to be a possible means of separating these pigments without resorting to fractional crystallization. JONES and ROSA (7) and HOWARD (5) mention only two pigments present in tomatoes, the red lycopersicin and the yellow carotin. Since the lycopersicin and carotin in the petroleum ether solution were not separated, the results are expressed as milligrams of carotinoid per 1,000 grams of fruit on the assumption that the two isomers may exert the same effect.

It was necessary to discontinue the use of the pyridine method because of the poisonous effects of the fumes. The remaining fruit was analyzed by a modification of the LUBIMENKO extraction method. This method consisted of extracting the pigments from the finely ground pulp with hot 95 per cent. ethyl alcohol; the alcoholic extractions in a separatory funnel were cooled and petroleum ether added in 50-cc. portions. The carotinoids were obtained in the petroleum ether layer by diluting the alcoholic extractions with water. The combined petroleum ether extracts were filtered through anhydrous sodium sulphate and the depth of color determined as in the pyridine method. These two methods agree within 0.001 mg. per gram of fruit using the WILLSTÄTTER standard.

The moisture content of the fruit samples was determined by drying to constant weight *in vacuo* at 58° C. The pH of the fruit juices was obtained by the HILDEBRAND electrometric set up as described by CLARK (1).

Results

TREE FRUITS

None of the bagged fruits developed the characteristic blush on the cheek. The skin was thinner and slipped more easily from the flesh, and the Elberta and Mayflower peaches were almost entirely devoid of the characteristic fuzz. The flesh was firm, separated easily from the pit and

had the same flavor as the unbagged fruit. No difference in size of the bagged and unbagged fruits was noticeable. On removal from cold storage, all the fruits were in good condition, but on exposure to air the bagged white peaches did not oxidize as rapidly as the unbagged. This difference was observed in all other cases of bagged fruits, but none remained unoxidized in the air as long as the bagged white peaches. Table I gives the results of the determinations of moisture, pH and carotinoid content of the fruits.

TABLE I
MOISTURE, pH AND CAROTINOID CONTENT OF THE TREE FRUITS

FRUIT AND VARIETY	COLOR OF FLESH	LIGHT CONDITIONS	MOISTURE	pH	CAROTINOID PER 1,000 GM. FRUIT
			<i>per cent.</i>		<i>mg.</i>
Peach, Elberta	yellow	bagged 3/31/28		4.31	11.0*
“ “	“	bagged 6/9/28	85.6	..	8.2
“ “	“	unbagged	85.8	.	1.9
“ Mayflower	white	bagged 3/31/28		4.50	no color
“ “	“	unbagged		4.39	no color
Nectarine, Humboldt	yellow	bagged 3/31/28	83.3	4.02	5.9
“ “	“	unbagged	87.3	4.22	7.2
Nectarine, Stanwick	white	bagged 3/31/28		4.51	no color
“ “	“	bagged 5/10/28	82.9	4.06	no color
“ “	“	bagged 6/9/28	80.4	4.14	no color
“ “	“	unbagged	79.7	4.17	no color
Apricot, Royal	yellow	bagged 3/31/28	85.9	4.17	8.4
“ “	“	unbagged	82.2	4.13	21.7

* These figures are averages of from 2 to 10 separate determinations.

The petroleum ether soluble pigments are calculated as carotinoids although in no case was xanthophyll found in appreciable quantities. The absence of light appears to favor the development or maintenance of the

carotinoids in the yellow Elberta peach as the bagged fruits have higher carotinoid content than the unbagged and the earlier they are bagged the higher the content of these pigments. This is not true with the other yellow fruits (Humboldt nectarine and Royal apricot) since the absence of light decreases the content of the fat soluble pigments. The carotinoid content of both bagged and unbagged fruits of the white fleshed nectarine and peach was not great enough to be measured by the method used.

GUTHRIE (4) found that with tomato and soybean plants grown in the dark several days, the chlorophyll content of the leaves decreased but the carotinoids remained the same. He found also a large increase in the carotin-xanthophyll ratio, which appeared to be due to a decrease in xanthophyll and an increase in carotin. If there is a change of xanthophyll to carotin this may affect the colorimetric readings in which all of the carotinoids are measured as a group and not individually. GUTHRIE also showed that reducing the light intensity to 12 per cent. of normal sunlight resulted in an increase in chlorophyll and carotinoids.

Perhaps the factors which govern the carotinoid formation or destruction in one variety of fruit are more readily catalyzed or depressed by light changes than in the others. In some varieties of fruits, light or certain wave lengths of light, may exert a destructive influence on the carotinoids during their formation or destroy them after they are formed. The exclusion of these light waves by bagging may therefore result in a higher carotinoid content, as was found in the Elberta peach. It was also shown that in some of the fruits, especially the peaches, the bagged fruits when exposed to the air, oxidized much more slowly than the unbagged fruits. If this oxidation destroys some of the pigment, bagging the fruits would result in a higher carotinoid content, other factors being equal.

TOMATO FRUITS

No differences were detected in the flavor or texture of the bagged and unbagged fruits. The mature bagged fruit was more evenly colored than the unbagged fruit. During the ripening, there was no evidence of chlorophyll formation, the fruit being pure white and gradually shading into the yellow or red as it approached maturity, indicating that chlorophyll formation in the fruit is not necessary for the normal coloring of tomato fruits. KRAUS (8), who was the first to work along this line, sought to show that the yellow color of the tomato and the rose was originally green, and that the yellow color originated from the green, also that the green chlorophyll-containing bodies, with or without a continuation of form, acquired a yellow color. The TOBLER'S (17), working with the fruit of *Momordica balsamina*, decided that throughout the ripening process the formation of carotin was

connected with the decomposition of chlorophyll. LUBIMENKO (9) believed that lycopersicin appears after the decomposition of the chlorophyll. He states that lycopersicin does not exist within the chloroleucites of the fruit before the decomposition of chlorophyll. Later (10) the same author found during the conversion of chloroplasts into chromoplasts, that chlorophyll and the accompanying pigments were decomposed, yellow pigments accumulated and then underwent alterations. He maintained that light was no longer necessary for the formation of lycopersicin after the chlorophyll had been formed. The present work shows that both light and chlorophyll, directly are unnecessary for the formation of lycopersicin in the fruit.

DUGGAR (2) found that the development of lycopersicin in the tomato is preceded by the paling and ultimate disappearance of the chlorophyll and that a yellowish or orange cast followed in the chloroplastids. Mature bagged fruit of Clark's Albino was almost white with a faint tinge of yellow instead of the deeper yellow of the unbagged fruit while the deposits of color of Ruby Gold were a deep red instead of the deep yellow of the unbagged fruit, indicating a probable greater increase of lycopersicin than of carotin. There was no apparent difference in the color of the mature bagged and unbagged fruit of the red fleshed varieties although the latter passed through the chlorophyll stage while the former remained white in the immature stage. Table II shows the results of the determinations of moisture, pH and carotinoid content of the fruits.

The five varieties used in these analyses can be separated into two groups with respect to their carotinoid content accompanying changes in exposure to light. Clark's Albino, having pale yellow flesh and Ruby Gold, with deep yellow flesh, show an increase in carotinoid content of the bagged over the unbagged fruits. Both of these varieties have colorless skins. Especially with Clark's Albino it appears that light may exert a destructive influence on the pigment content of the fruits, the mature green and ethylene ripened (in the dark) fruits having a greater carotinoid content than those exposed to light until maturity. WIESNER (18) observed that potato sprouts which formed in the light showed little if any yellow pigment while those formed in the dark developed from 30 to 150 per cent. more pigment. ELFVING (3) and IMMENDORFF (6) found that carotinoids increased greatly in leaves under conditions which depressed chlorophyll formation, that is, low temperature and very diffuse light. The fruits of Clark's Albino grown in the greenhouse, resulting in reduced light and with the shorter wave lengths removed, also had higher content of the carotinoids than those grown outside and exposed to the full sunlight. In the second group, composed of Globe, having red flesh, California Earliana, of medium red flesh and Gigante Ingrengnoli with deep red flesh the unbagged fruits, both outdoor and greenhouse grown, have a higher carotinoid

TABLE II
MOISTURE, PH AND CAROTINOID CONTENT OF TOMATO FRUITS

VARIETY AND NORMAL COLOR	TREATMENT	MOISTURE	PH	CAROTINOID PER 1,000 GRAMS OF FRUIT	COLOR STANDARD USED
Clark's Albino Pale yellow flesh Colorless skin	Outdoor grown, mature green	<i>per cent.</i> 92.4	4.43	<i>mg.</i> 0.23*	Sprague
	" " " ripe unbagged	93.6	4.27	no color	
	" " " ripe bagged	91.7	—	too dilute to read	—
	" " " ethylene ripened	93.6	4.39	0.56	Sprague
Ruby Gold Deep yellow flesh Colorless skin	Greenhouse grown, unbagged ripe	91.8	4.19	0.29	"
	" " " ripe bagged	—	4.17	2.50	"
	Outdoor grown, ripe unbagged	92.5	4.43	3.40	"
	Greenhouse grown, ripe unbagged	92.1	3.72	0.30	Willstätter
	" " " bagged	88.5	4.18	3.25	"
Globe Red flesh Pale yellow skin	Outdoor grown, ripe unbagged	90.9	4.23	3.45	"
	" " " bagged	91.5	4.27	2.50	"
	Greenhouse grown, ripe unbagged	88.7	4.07	3.45	"
	" " " ripe bagged	90.4	4.18	1.75	"
	" " " mature green	89.8	4.31	1.10	Sprague
	" " " one-half ripe	—	4.00	1.40	Willstätter
	" " " three-fourths ripe	—	4.23	1.40	"
California Earliana Medium red flesh Orange yellow skin	Outdoor grown, ripe unbagged	90.8	4.24	5.30	"
	Greenhouse grown, ripe unbagged	90.2	4.30	2.15	"
Gigante Ingregnoli Deep red flesh Orange yellow skin	Outdoor grown, mature green	91.8	4.26	3.20	Sprague
	" " " ripe unbagged	91.8	4.26	3.05	Willstätter
	" " " bagged	90.8	4.38	1.80	"
	" " " ethylene ripened	91.2	4.19	6.30	"
	Greenhouse grown, ripe unbagged	90.6	4.15	5.15	"
	" " " ripe bagged	90.9	4.49	1.40	"

* These figures are averages of from 2 to 10 separate determinations.

content than the bagged fruits, suggesting that light is directly related to carotinoid development or preservation in these varieties. The varieties of this group have colored skins although that of the Globe variety is very pale yellow and often classified as colorless.

The fruit of the Globe variety analyzed at the various stages of immaturity had low carotinoid content. In only one case (Gigante Ingrenoli) was the carotinoid content of greenhouse grown fruits higher than those grown outside. The differences in the hydrogen-ion concentrations of bagged and unbagged fruits also should receive consideration. With one exception, Clark's Albino, greenhouse grown, where there is but 0.02 pH difference, the bagged fruits have a higher pH than the unbagged ones. It appears highly probable that these changes in hydrogen-ion activity may affect the enzyme activity and other metabolic action of the fruit which would alter their pigment content. The differences in carotinoid content between greenhouse and outdoor fruits grown at Berkeley, where very little ultraviolet rays reach the outdoor grown plants, may be altered if grown where more of these rays reached the plants. However, light on the fruit is unnecessary for the production of carotinoids in those fruits.

DUGGAR (2), using green fruits which had been exposed to light until almost ready to turn color, showed that light was unnecessary for the normal pigmentation of red tomatoes.

It is possible that part of the variation in the pigment content reading was due to the changing relative amounts of carotin and lycopersicin present which were extracted and determined unseparated. WILLSTÄTTER and ESCHER (19) and MONTEVERDE and LUBIMENKO (11) have shown that the principal pigment in the tomato is lycopersicin although carotin is present in small amounts.

Summary

1. A new method is described for the extraction of the carotinoid pigments from fresh tissue using pyridine (b. p. 112–115° C.) as the solvent.

2. Peaches, apricots and nectarines bagged in the blossom stage and developing in total darkness do not oxidize as rapidly when exposed to the air as those developing in full light.

3. The bagged Elberta peach developed a higher carotinoid content than the unbagged while the reverse was true of the Humboldt nectarine and Royal apricot. The Mayflower, white fleshed peach, and Stanwick, white fleshed nectarine, contained such small quantities of the carotinoids that no quantitative determination could be made.

4. During the ripening of tomato fruits in total darkness no chlorophyll developed. The fruits were pure white and gradually shaded into the yellow or red as they approached maturity. When mature, the bagged and unbagged fruits of the red varieties were the same color.

5. Mature bagged fruit of Clark's Albino was almost white with a faint tinge of yellow instead of the deeper yellow of the unbagged fruit. The localized deposits of color of Ruby Gold were a deep red instead of the deep yellow of the unbagged fruit.

6. Fruits of Clark's Albino and Ruby Gold, having pale yellow and deep yellow flesh, respectively, show an increase in carotinoid content of the bagged over the unbagged. Fruits of Clark's Albino grown in the greenhouse also had a higher content of carotinoids than those grown outside.

7. Both the outdoor and the greenhouse grown fruits of the three red fleshed varieties had a lower carotinoid content when bagged.

8. With one exception, greenhouse grown Clark's Albino where there was but 0.02 pH difference, the bagged fruit had a higher pH than the unbagged fruit.

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STUDIES ON INANITION IN *ARACHIS* AND *PHASEOLUS*

G. M. SHEAR

(WITH FOUR FIGURES)

I. Introduction and literature

The changes that occur in organisms during starvation or inanition have been the subject of many investigations by animal and human physiologists, but very little has been done with plants, and the botanical literature on this subject is consequently very meager. Even workers on the animal side, JACKSON (7) and HOWE (5), have noted this and have expressed the desirability of a more thorough understanding of the effects of inanition on plants. It is only through studies of many organisms under varying conditions that we may hope to get at the fundamental likenesses and differences in the metabolism of plants and animals. The purpose of this paper is to add to our knowledge of the changes that take place in plants during inanition, induced by the checking and removal of the food supply stored in the cotyledons.

Inanition in its broadest sense refers to the subsistence of an organism on one or more of the essential mineral or organic substances contained within its body. MORGULIS (13) divides inanition into three main types, physiological, pathological, and experimental. The first refers to a normal condition in the life cycle of an organism, such as hibernation in animals. The second is the result of an organic derangement produced by some disease. The last is the withholding of nutriment, generally for the purpose of scientific studies. The latter two produce very marked changes from the normal functioning of the organism, the extent of these depending on the severity of the inanition. JACKSON (7), on the other hand, divides inanition into total or quantitative and partial or qualitative, each of these groups being, in turn, divided into complete and incomplete inanition. He considers plants growing in distilled water in the absence of light as being subjected to conditions of total inanition.

Starvation considered from the most widely accepted standpoint consists merely of the withholding of organic matter from an organism and the author uses it in that sense. The availability of inorganic salts is of little or no direct consequence in prolonging life in the absence of food. Therefore unless an organism has an excessive amount of food stored up, the salts which it contains will be sufficient to more than supply its needs until it dies from lack of food, or from a disruption of its metabolic processes due to the depletion of its food supply. From a physiological point of view however, an organism is not actually starving until its food supply

has been depleted to an extent that causes a derangement of its normal metabolic functions. A seedling growing in the dark in the absence of extraneous organic material grows normally until it is well above the medium in which it is planted. PEARL, WINSOR, and MINER (16) found that the rate of increase in the length of shoots of muskmelon seedlings grown in the dark on agar at 30° C. was very rapid for about ten days. This was followed by a rapid falling off lasting about two days, after which elongation ceased. Starvation as effecting shoot elongation made its first appearance in approximately ten days. These first stages of growth in seedlings might be classified under the heading of physiological inanition as given by MORGULIS (13).

In discussing fasting HOWE (5) says that the fat available in an organism exerts a marked effect upon the metabolism. "So long as there is sufficient fat in the organism to supply the energy requirements, the protein metabolism will remain at a minimum. When, however, the fat deposits are depleted, the body is forced to use protein to furnish the necessary energy. The result is a more rapid protein consumption and an early death."

According to JACKSON (6), plants, much more than animals, appear susceptible to modification by various external factors including the food supply. Sooner or later during inanition in all living organisms the cells pass from a stage of simple atrophy into a condition of degeneration. The recovery is thus seen to be dependent upon the progress of inanition.

HOTTES (4) in his cytological studies of starving seedlings of the broad bean, *Vicia faba*, found that there was an increase in vacuolization and that the cytoplasm became so attenuated that it was barely visible as a narrow band in close contact with the cell wall. The quantity of chromatin also was reduced. He found that the primordia of the secondary roots ceased development, as the food is utilized by the active cells at the tip of the main root.

COUPIN (2) found that seedlings grown in distilled water in darkness lived various lengths of time. He reports that seedlings of the nut pine grown in this way lived 60 days; those of pumpkin 46 days; of winter vetch, 44 days; of lentil, 40 days; of marvel of Peru, 39 days; of pea, 33 days; of bean, 32 days; of sunflower, 30 days; of buckwheat, 25 days; of radish, 24 days; of nasturtium, 23 days; of spinach, 22 days; of tomato, 21 days; of beet, 20 days; of common cress, and mustard, 18 days. He attributes this "resistance to inanition" as being due especially to the quantity and quality of the reserve material in the seed.

BOUYGUES (1) and VON PORTHEIM (17) have studied certain phases of starvation in the seedlings of *Phaseolus vulgaris*. Both workers found that seedlings without cotyledons developed into much smaller and weaker plants

than those with cotyledons. The former claims that there is a balance maintained between the root and shoot of the check and starving seedlings. The latter found that the length and number of internodes developing corresponds to the reserve material, originally at the disposal of the seedlings. He noted a growth acceleration in the hypocotyl of seedlings with one or part of both cotyledons removed, which he attributed not to a stimulation from wounding, but to nutritional disturbances brought about by the lowering of the amount of reserve food. This phenomenon has been given the name hunger etiolation by NOLL (14).

II. Materials and methods

Seedlings and embryos of the Spanish peanut, *Arachis hypogaea*, and seedlings of the common garden bean, *Phaseolus vulgaris*, were used.

Seeds weighing more than 0.550 grams were treated for forty-five minutes with a 0.025 per cent. solution of Uspulun, and from them the cotyledons were removed in a steamed cabinet to insure sterile conditions. The embryos were placed in test tubes containing approximately 15 cc. of 1.5 per cent. sterile agar. The tubes were kept in a dark room at an average temperature of 22° C. At two day intervals sets of four embryos were placed five feet beneath an 1834 candle power light to test for chlorophyll formation. The intensity of the chlorophyll was determined at the end of the first and second days of exposure, by a comparison with RIDGEWAY'S color charts (20).

The seeds used in the growing of seedlings were selected so as to fall within five per cent. of the average air dry weight of a random sample of seeds. This was 0.500 to 0.550 grams for peanuts and 0.400 to 0.440 grams for beans. These seeds were kept in a covered can in a desiccator without a drying agent so as to maintain the original moisture content. Samples of these seeds were dried and the moisture content and seed-coat weight determined. The average of these two was for peanuts 33 ± 0.435 and for beans 74 ± 0.672 mg. From these the calculated dry weight and seed-coat weight of each individual seed was determined.

Seedlings for the determination of chlorophyll formation were grown in glass jars in pure silica sand, six seedlings to a jar. These were grown at the same temperature as the embryos. The cotyledons were removed from half of the seedlings after six days. At two day intervals until chlorophyll ceased to form in the starving seedlings, one jar of each with a check jar was placed beneath an 1834 candle power light. The tops of the seedlings were approximately four feet from the light. Determinations of the chlorophyll intensity were made at the end of one and two days, as with the embryos. The seedlings being susceptible to injury from excessive transpiration had to be kept at a humidity above that of the room. This

was accomplished by placing the jars in which the seedlings were growing in a tub, the bottom of which was kept covered with water, and over this another tub was inverted. The jars of plants under the light were also placed in a tub containing water.

In the experiments to determine loss of weight during inanition, weight records of individual seeds were kept. The seedlings were grown in glass jars filled with pure silica sand containing 50 per cent. of its water holding capacity. Eight jars containing a total of thirty seeds constituted a set and were grown in a wash boiler with a ventilated top. All sets were grown at 23° C. and the cotyledons were removed from half at the end of six days. They were then grown for different lengths of time, some sets at 23° C., others at 30° C.

To prevent undue oxidation of the peanut oil at high temperatures, all of the drying was done in a vacuum oven at 75° C. until a constant weight was reached.

Some difficulty was encountered in securing the dry weight of roots of seedlings grown in quartz sand. It was found impossible to remove all of the sand from the roots by merely washing them. It was therefore necessary to get the weight including the sand which adhered to them, then burn the material, wash off the ash, and get the weight of the sand. In many instances the sand weighed more than the roots. Some investigators have ignored the sand that adheres after washing and consequently have introduced a big source of error.

Ether extract determinations by means of a Soxhlet extractor were made on starving and check seedlings of the peanut. These were grown at 23° C. as in the preceding experiments and determinations made on groups of ten plants at two day intervals for a period of from six to eighteen days. The seedlings were dried, ground in a mortar, and then dried again for several hours before weighing. Porous bottomed crucibles were used to hold the material, instead of paper extraction thimbles as they are much easier to handle and the chances for error in weighing are less. After extraction for twelve hours the material was dried and reweighed.

III. Results and discussion

CHLOROPHYLL FORMATION AND GROWTH OF PEANUT EMBRYOS

It is a well-known fact that many plant embryos have enough inherent vitality to grow and form chlorophyll. The length of time that this will occur after growing in the dark, and the intensity of the greening, are indications of physiological readjustments to meet the demands of growth and maintenance.

From the twelfth day of growth through the twentieth day there is a gradual decrease in the intensity of the greening characterized by changes

TABLE I
INTENSITY OF CHLOROPHYLL IN PEANUT EMBRYOS

GROWTH PERIOD	TIME IN LIGHT	COLOR
<i>days</i>	<i>hours</i>	
10	24	Scheele's green
	48	Scheele's green
12-14	24	Green-yellow
	48	Scheele's green
16	24	Viridine green
	48	Scheele's green
18	24	Viridine green
	48	1 Scheele's green 3 Viridine green
20	24	Light viridine green
	48	Viridine green
22-35	24	Pale cendre green
	48	Viridine green
38	24	Pale cendre green 1 no chlorophyll
	48	Viridine green 1 no chlorophyll

in both hue and tone (table I). The hue becomes greener and the tone lighter. A gradual decrease in the intensity of the yellow could be noted at the time the embryos were placed in the light. This decrease in yellow pigment, probably carotin, was responsible for the change in hue. From the twentieth to the thirty-eighth day at which time the embryos start losing their ability to form chlorophyll the rate of greening remains constant. At first the epicotyl and leaflets turn green, but there is a gradual decrease in the area of greening in the leaflets from their tip to base, and in the final stages of chlorophyll formation only the epicotyl and base of the leaflets turn green.

The embryos practically cease elongation after four weeks. Measurements taken on twenty-six of them after thirty days gave an average shoot length of 2.0 cm. and a root length of 5.9 cm. The shoot length represents mostly hypocotyl as the epicotyl is not more than 1 or 2 mm. in length. Most of the embryos produce one to several secondary roots which show their great vigor. As shown by HOTTES in *Vicia faba* and by some preliminary work of the author with beans, peas, pumpkin, and squash, the removal of the cotyledons during germination and before the secondary

root primordia have reached too advanced a stage, inhibits their further development.

The embryos with excised cotyledons grown to find out how long they were capable of living, probably died from desiccation rather than inanition. One, however, lived for one hundred and forty days before the agar dried up. This, when compared with the length of life, (twenty some days), of peanut seedlings whose cotyledons were removed after six days' growth, shows that the rate of metabolism at the time that starvation is induced has a marked effect on the length of life. The embryos are able to utilize their small reserve of food much more economically than seedlings with a much greater reserve. From these results it would seem that Howe's (5) statement, "adult organisms can fast longer than the young of the same species" does not necessarily hold for plants.

CHLOROPHYLL FORMATION IN SEEDLINGS OF THE PEANUT AND BEAN

The intensity of greening at different periods of growth is shown in table II. As in the embryos there is a decrease in the amount of yellow pigment in the starving seedlings. After seventeen days in the case of the beans and nineteen for peanuts with the cotyledons removed, chlorophyll is no longer formed or at least not in quantities large enough to be detected by the method used. With the advance of inanition it is always the young-

TABLE II
INTENSITY OF CHLOROPHYLL FORMATION IN SEEDLINGS

GROWTH PERIOD	LIGHT	COLOR, COTYLEDONS REMOVED	COLOR, CHECK
PEANUTS			
<i>days</i>	<i>hours</i>		
15	24	Viridine green	Scheele's green
	48	Scheele's green	Cossack green
17	24	Light yellow-green	Scheele's green
	48	Green-yellow	Grass green
19	24	Pale greenish-yellow	Scheele's green
	48	Pale greenish-yellow	Grass green
	72	Pale greenish-yellow	Grass green
BEANS			
15	24	Light yellow-green	Yellow-green
	48	Colliste green	Scheele's green
17	24	Light greenish-yellow	Green-yellow
	48	Light greenish-yellow	Scheele's green
19	24	Light greenish-yellow	Scheele's green
	48	Light greenish-yellow	Scheele's green
	72	Light greenish-yellow	Scheele's green

est leaves that show the most intense greening, and that retain the power of forming chlorophyll the longest. The older leaves gradually cease forming chlorophyll from their outer edges toward the base. In order to obtain as much uniformity as possible all color determinations were made from the youngest leaves.

As PALLADIN (15) states one of the prerequisites for chlorophyll formation is the presence of soluble carbohydrates. The ability of the seedlings to form chlorophyll and the amount of chlorophyll formed are undoubtedly an index of the available carbohydrates and therefore indicate the progress of starvation. This is substantiated by the fact that the older leaves failed to form chlorophyll while the younger ones were still capable of doing so. The soluble carbohydrates would be used up in the older leaves or transported to the younger ones, thus depleting their supply first.

GROWTH OF PEANUT AND BEAN SEEDLINGS

As stated in the methods, thirty seeds were planted in each set, half of them to be used as checks. This was done to assure at least ten plants for each group. Little difficulty was experienced with peanuts as can be seen from the number of seedlings in each group. Most of the loss came through lack of germination. The greatest difficulty experienced with beans was the tendency for them to come up with one or both cotyledons broken off. The main root in some of the beans could not free itself of the seed-coat, thus making the seedlings worthless. A few seedlings because of their abnormal growth were discarded.

Six days after planting was selected as the time for the removal of the cotyledons from the seedlings to be starved, since at that time the cotyledons of the peanuts were just above the surface of the sand. The dry weights obtained from the starving plants show that there is considerable variation in the actual weight of the seedlings from different sets at the time of cotyledon removal. This makes direct comparisons of different sets difficult in some cases although the relationships expressed in percentages negates such variations to a great extent.

Beans elongate more rapidly than peanuts during the first six days of growth (tables III and IV), although the average dry weight of the seedlings is practically the same. This means that the peanuts are much more stocky than the beans. This stockiness is due to the thick cortex of the hypocotyl. WALDRON (21) in his work on the peanut says that the hypocotyl often becomes thick and fleshy in its cortex. "This is more marked when growth is retarded from some cause and then the lower end becomes tuberous from a deposition of sugar. The roots of such are not able to utilize the food as fast as it is supplied from the seed." That the cortex of the hypocotyl is a reservoir of available food for the seedling is clearly

shown in fig. 2 by the wrinkled and withered condition of this region after the food has been utilized during inanition. This wrinkling occurs progressively from the upper portion of the hypocotyl toward the base.

The secondary roots of the peanut were less than a centimeter long at the time of cotyledon removal, whereas, in the bean there were usually four secondaries that were several centimeters long. During the course of inanition the secondaries of the peanut increased in number and the first ones reached a length of from 2 to 3 centimeters, (fig. 1). In the bean the four original secondaries more than doubled in length while none of the others developed far beyond the primordial stage.

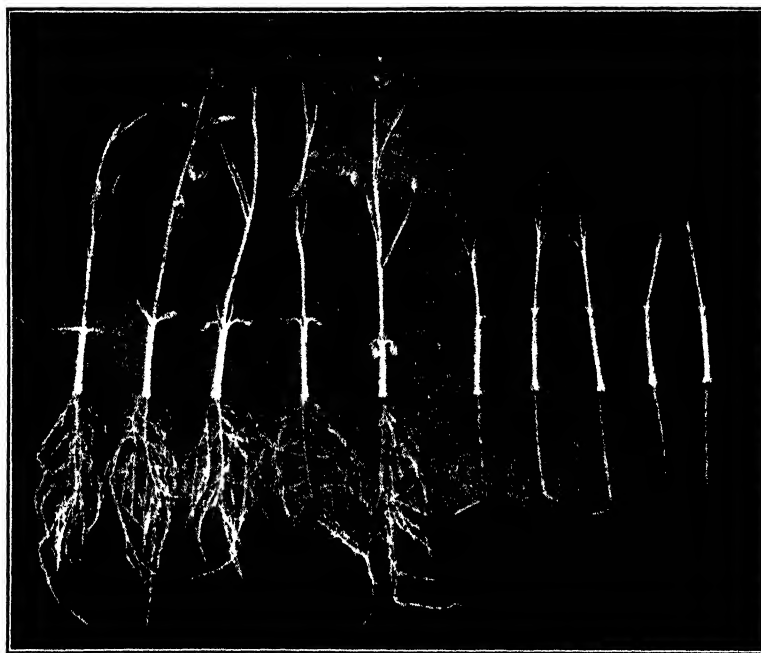


FIG. 1. Peanut seedlings after eighteen days growth at 23° C. Check plants at the left, at the right plants from which the cotyledons were removed after six days.

The general appearance of the starving plants was found to agree with the observations of VON PORTHEIM (17) and BOUYGUES (1). They were smaller, developed fewer and shorter internodes, and produced fewer and smaller leaves. The hypocotyls of the starving peanuts were slightly longer than were those of the checks (fig. 2). VON PORTHEIM found this to be true of bean seedlings with one or parts of two cotyledons removed, although, as confirmed by the author, beans with both cotyledons removed did not show this phenomenon.

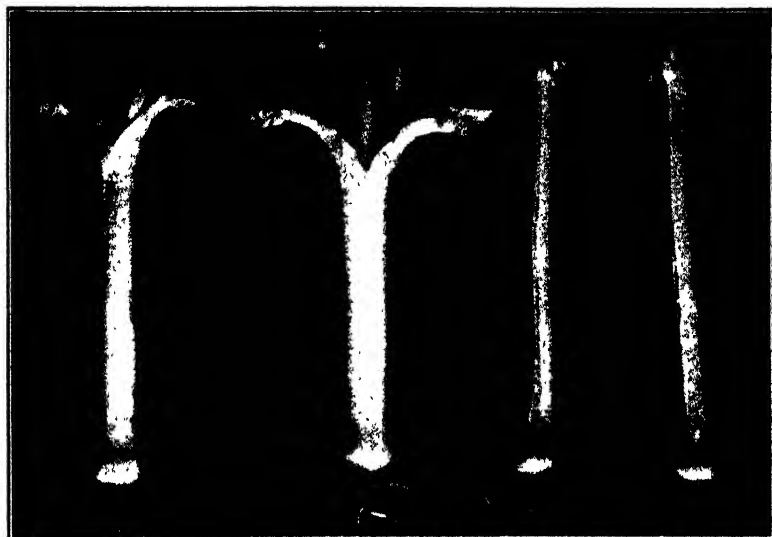


FIG. 2. A detailed view of the hypocotyls of four of the seedlings shown in figure 1. Note the wrinkling and etiolation of the starving seedlings at the right.

The ability to adjust themselves to abnormal conditions seemed to be greater in the starving peanuts than in the checks. The check seedlings growing at 23° C. began developing translucent (watery) areas around the outer edge of the oldest leaves after fourteen days. These areas continued to enlarge and were apparently due to a "water logged" condition of the tissues. Upon exposing such plants to the dryer air of the laboratory these areas dried and turned brown, showing that the cells in such areas were dead. The starving seedlings failed to show this injury and were in good condition upon removal. Peanuts grown eight days at 30° C., after the removal of the cotyledons from half of the plants, showed a different type of injury. On the fifth day at 30° C. the terminal buds of all but two of the checks were dead and appeared black, whereas the starving plants showed no injury. At the time of removal of the set the terminal buds of the starving seedlings appeared straw colored and watery.

With regard to elongation in the peanuts, the roots seem to be the first to be affected by inanition. Even on the tenth day they were much shorter than those of the checks (table III). The shoots elongate at approximately the same rate through the tenth day, after which the checks greatly outclass the experimental plants.

The increase in seedling weight of the checks, (cotyledons excluded), was very rapid through the fourteenth day, after which the rate of transfer of food from the cotyledons was practically balanced by the loss through

TABLE III
GROWTH OF PEANUT SEEDLINGS

NO. OF PLANTS	GROWTH PERIOD	STARVED	AVERAGE CALCULATED DRY WEIGHT OF SEED MINUS SEEDCOAT	AVERAGE DRY WEIGHT OF COTYLEDONS	AVERAGE DRY WEIGHT OF SHOOT	AVERAGE DRY WEIGHT OF ROOT	TOTAL WEIGHT OF SEEDLINGS	AVERAGE LOSS IN WEIGHT	AVERAGE LENGTH OF SHOOT	AVERAGE LENGTH OF ROOT	AVERAGE TOTAL LENGTH
	days	days	gm.	gm.	gm.	gm.	gm.	gm.	cm.	cm.	cm.
A. At 23° C.											
12	6	check	0.496	0.386	0.085	0.021	0.106	0.003	3.6	8.5	12.0
12	10	check	0.495	0.219	0.214	0.061	0.276	0.000	10.9	16.0	26.9
12	10	4	0.494	0.367	0.086	0.023	0.109	0.016	10.0	10.7	20.7
12	14	check	0.498	0.086	0.275	0.095	0.368	0.042	22.0	21.4	43.4
12	14	8	0.494	0.362	0.085	0.023	0.107	0.024	15.1	12.1	27.2
14	18	check	0.498	0.055	0.274	0.099	0.372	0.070	28.8	22.9	51.7
12	18	12	0.502	0.348	0.093	0.028	0.122	0.032	19.0	14.8	33.8
B. At 30° C. from the sixth day of growth—											
14	10	check	0.495	0.092	0.259	0.100	0.359	0.045	19.7	23.5	43.2
12	10	4	0.491	0.354	0.086	0.024	0.110	0.026	14.6	15.1	29.7

respiration during the next four days. The cotyledons still contained a small amount of food on the eighteenth day. Due to variations in the weight at the time of cotyledon removal no direct comparisons of the starving plants can be made.

After six days' growth the peanut seedlings showed an average loss in weight, (based on the calculated dry weight of the seed minus the average seed-coat weight), of 3 mg. (table III). The loss in weight of the seedlings without cotyledons showed a steady increase, while the average loss for the checks at ten days was 0 mg. From this time on to the end of the experiment the checks showed a very rapid loss. Results for check seedlings between those of six and ten days gave an average gain of 4 mg. At this time the plants were quite large and the gain would have to be proportionally large to offset the loss through respiration. A gain in weight of seedlings from oily seeds has been recorded by several investigators. As early as 1855 HELLRIEGEL (3) detected an increase in the weight of rape seedlings during the early stages of germination. JEGOROW (8) found that the oil content and also the dry matter of *Cucurbita maxima* seedlings had increased at the end of six days' growth.

This increase is undoubtedly due to the decomposition of the oily reserve which from RHINE'S (19) results seems to be necessary for translocation. This entails, first, hydrolysis, and then partial oxidation. In the first of these processes water is chemically combined at the rate of three molecules for every molecule of fat. Oxygen then combines with the fatty acids to form intermediate compounds such as sugar. These processes must have produced a large excess of readily available food between the sixth and tenth days of growth.

Peanuts starved four days at 30° C. show the same general tendency as those grown at 23° C. Their growth is more rapid and consequently they lose more weight. The checks show a much greater loss than check seedlings grown for the same period of time at 23° C. Because of the injury to the seedlings at this temperature, records on longer periods of growth could not be obtained.

The length of the shoots of check beans grown at 23° C. increased rapidly until the fourteenth day, after which it practically ceased (table IV). The shoots of starving seedlings showed the same tendency. The roots, however, in both groups showed very little elongation after the tenth day. The general tendency of elongation would therefore seem to be more dependent on the age of the bean seedlings than upon their food-supply. Peanuts did not show this.

The changes in weight of the seedlings (exclusive of the cotyledons) was of necessity quite different as the checks will continue to increase as long as they can get sufficient food from the cotyledons, while the others

TABLE IV
GROWTH OF BEAN SEEDLINGS

NO. OF PLANTS	GROWTH PERIOD	STARVED	AVERAGE CALCULATED DRY WEIGHT OF SEED MINUS SEEDCOAT	AVERAGE DRY WEIGHT OF COTYLEDONS	AVERAGE DRY WEIGHT OF SHOOT	AVERAGE DRY WEIGHT OF ROOT	TOTAL WEIGHT OF SEEDLINGS	AVERAGE LOSS IN WEIGHT	AVERAGE LENGTH OF SHOOT	AVERAGE LENGTH OF ROOT	AVERAGE TOTAL LENGTH
A. At 23° C.											
	days		gm.	gm.	gm.	gm.	gm.	gm.	cm.	cm.	cm.
10	6	check	0.344	0.197	0.084	0.020	0.104	0.043	9.9	14.2	24.1
10	10	check	0.339	0.034	0.174	0.037	0.211	0.094	29.3	27.8	57.1
11	10	4	0.346	0.208	0.073	0.020	0.094	0.044	20.3	20.6	40.9
10	14	check	0.352	0.032	0.185	0.035	0.219	0.100	40.7	27.3	68.0
11	14	8	0.348	0.198	0.076	0.021	0.098	0.052	29.0	22.0	51.0
10	18	check	0.342	0.032	0.165	0.036	0.201	0.109	41.6	28.8	70.4
12	18	12	0.347	0.206	0.067	0.020	0.088	0.053	30.6	20.8	51.5
B. At 30° C. from the sixth day of growth—											
10	10	check	0.340	0.031	0.175	0.038	0.213	0.095	32.0	27.0	59.1
11	10	4	0.345	0.165	0.099	0.024	0.123	0.057	26.0	24.5	50.5
10	14	check	0.344	0.032	0.165	0.035	0.200	0.113	42.3	27.0	69.4
9	14	8	0.345	0.179	0.081	0.023	0.103	0.062	32.1	21.1	53.2

will start losing when their supply of food is removed. The root and shoot weights of the checks was found to be proportional to their lengths. The root and shoot weights of the starving plants show that the root weight remained fairly constant, while most of the loss was from the shoot.

The loss in weight of the checks was very rapid up to the tenth day and from then on it was much slower. This decrease in the rate of loss occurs at about the same time that the reserve food has been completely removed from the cotyledons and is an indication of inanition. The loss of weight in the starving plants only averaged 10 mg. in the twelve days of inanition as compared with 66 mg. loss for the checks during the same period.

Both check and starving beans grown for six days at 23° C. and then transferred to 30° C., show a greater and more rapid shoot elongation than do those that are not transferred, but that are grown at 23° C. (table IV). They maintain the same relationship to each other regardless of their more rapid growth.

The loss in weight of peanuts during the period of induced inanition at 23° C. is approximately three times as great as for beans during the same period. The checks of both on the other hand lost practically the same amount during this time although the beans lose most rapidly from the sixth to tenth days while the peanuts lose most from the tenth day on. The peanuts in spite of the loss of their cotyledons have enough reserve food in their hypocotyls to maintain a higher rate of metabolism than beans under similar circumstances. The food from the bean cotyledons is translocated at a slower rate than it is from the cotyledons of peanuts. This accounts for the accumulation in the hypocotyl.

That starvation upsets the normal ratio of root to shoot growth is clearly shown in figure 3, where the percentages of shoot weight and length to total weight and length are given. While weight is a more accurate measure of growth than length, these show a consistent correlation with more of a tendency for the curves to converge in the length measurements. A very significant difference in the reaction of peanuts and beans to inanition is shown by these data. In the peanuts the percentage of shoot weight and length to total weight and length is less for the starving seedlings than for the checks. This relationship is just the reverse for beans. When the results of the starving beans and peanuts with regard to percentage shoot weight to total weight are compared, as is shown in the following data,

Beans	80.6	78.2	78.4	76.1
Peanuts	80.5	78.5	78.8	76.8

a remarkable uniformity is seen. It is probable that through the removal of the cotyledons the carbohydrate-nitrogen ratio is interfered with. REID (18) has shown, that the root-shoot ratio of different kinds of seedlings grown in the dark is more or less dependent upon their carbohydrate-

nitrogen ratio. Seedlings with a large relative amount of carbohydrates produce proportionally greater root systems. It would be interesting to determine this ratio for starving seedlings of beans and peanuts grown as described to see whether the same carbohydrate-nitrogen ratio in different plants is indicated by their having the same root-shoot ratio.

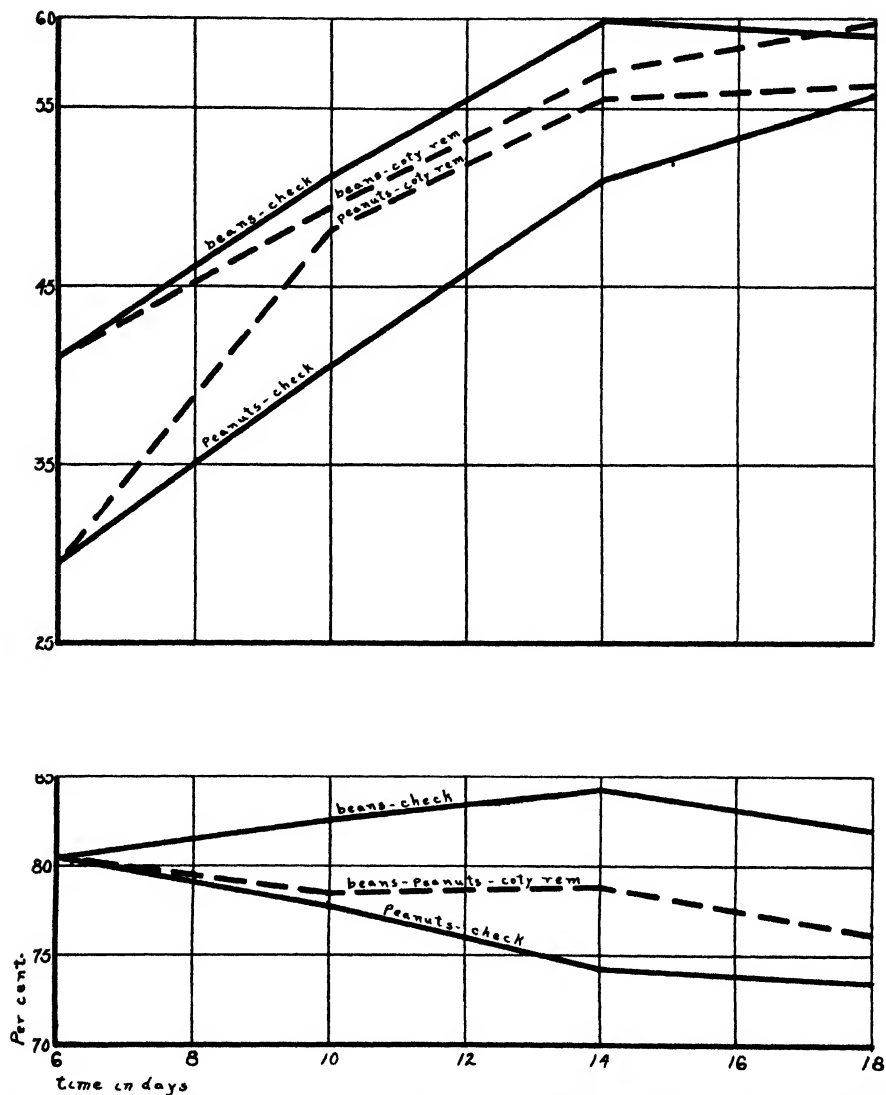


FIG. 3. Upper graph, per cent. shoot length to total length of peanut and bean seedlings grown at 23° C. Lower graph, per cent. shoot weight to total weight of peanut and bean seedlings grown at 23° C.

OIL CONTENT OF PEANUT SEEDLINGS

All of the determinations were made after discarding the cotyledons in order to make the checks comparable to the starving plants.

The results shown in figure 4 give the per cent. of oil, as ether extract, that is found in the seedlings at two day intervals of growth from the sixth

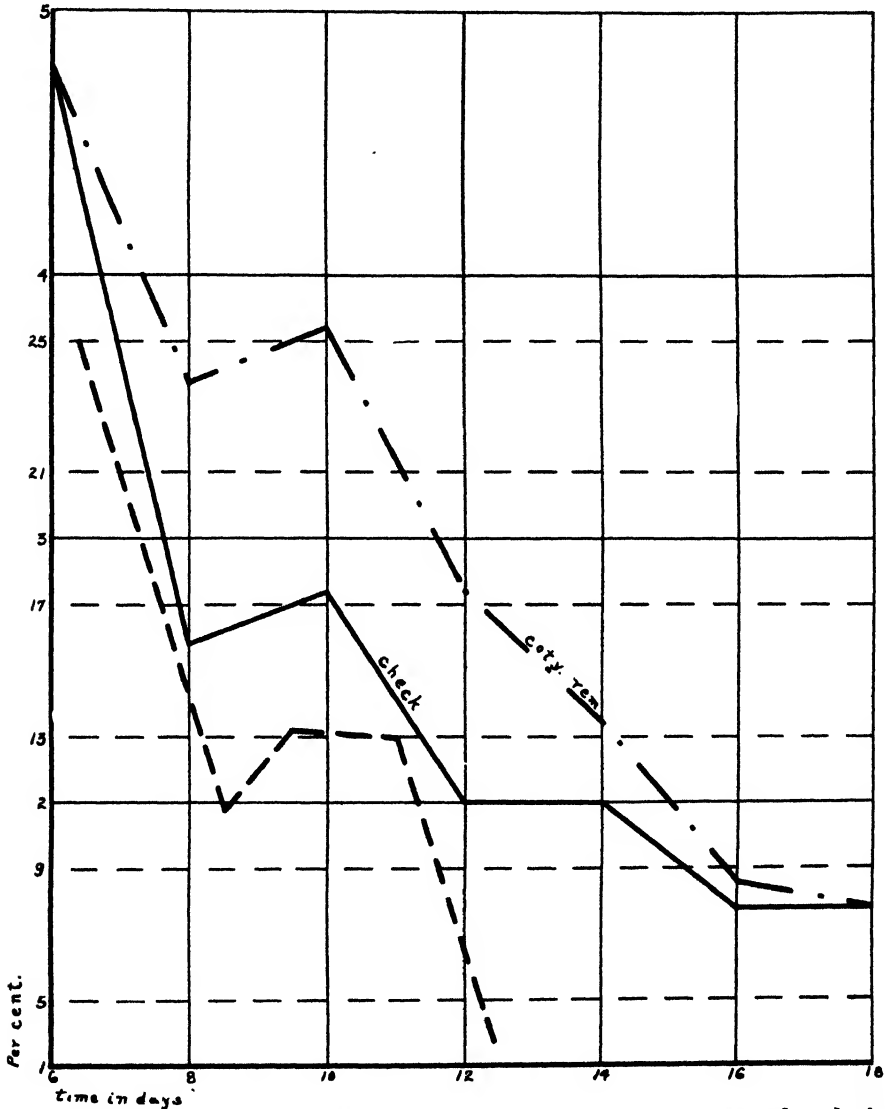


FIG. 4. Per cent. oil in peanuts grown at 23° C. The bottom curve, legend on broken lines, represents the per cent. of oil in sunflower seedlings, after MATTHES.

through the eighteenth day. A small percentage of the extract probably consists of cutin, and other ether soluble substances that are unavailable as food. This may explain the presence of over 1.5 per cent. extract from the starving seedlings at eighteen days when they have nearly reached the point of death. From the sixth to the eighth day the seedlings with their cotyledons removed show a marked decrease in oil content, followed by a slight rise during the next two days, after which there is a steady decrease up to the eighteenth day. Until the eighteenth day, the loss in percentage of oil in the check seedlings is more rapid than in the experimental plants. On the eighteenth day the per cent. is the same in both groups. As shown by figure 4, the loss shows a definite four day cycle, two days of decrease followed by two days during which the percentage remains practically constant. It is probable that the graph would not have shown as much regularity if different intervals of time had been used in making determinations. Shorter intervals would undoubtedly have given a very irregular curve as not all plants reach the same stage at the same time. The works of MAQUENNE (9) and MILLER (11) show the effect of too long and irregular intervals of time upon oil determinations. MAQUENNE found the following percentages of oil in peanuts during germination: 51.39 at 0 days, 49.81 at 6 days, 36.19 at 10 days, 29.00 at 12 days, 20.45 at 18 days, and 12.16 at 28 days. MILLER obtained the following results with sunflowers: 9.9 at 3.5 days, 7.4 at 5 days, 3.8 at 7 days, 3.1 at 10 days, and 1.8 at 14 days. He (12) says that the per cent. of ether extract falls very rapidly, and after the cotyledons are above ground it remains almost constant.

The more recent work of MATTHES (10), part of which is represented in figure 4, shows that sunflowers have the same tendency as peanuts to give a decrease in the percentage of oil followed by a slight increase. It is also interesting to note that although the percentage of oil is higher in his determinations, due to his including the cotyledons, the time and intervals of fluctuation practically coincide with the results for peanuts. He also found a rather regular rise and fall in the percentage of some of the intermediate products of the decomposing oil.

These results show clearly that the rate of oil consumption which should be a good index of metabolism is markedly reduced in seedlings with their cotyledons detached. During the early stages before the inanition becomes severe the oil pattern is the same as for the seedlings with an abundant food supply. Although they are unable to maintain this uniformity, they show themselves to be more conservative in the use of the oil at their disposal.

IV. Summary and conclusions

1. Peanut embryos growing at room temperature in darkness on sterile agar are capable of living more than four months. They cease elongation

in about four weeks and lose their ability to form chlorophyll after six weeks.

2. Embryos of peanuts live longer and form chlorophyll for a longer time than seedlings of similar plants in which inanition has been induced by removal of the cotyledons after active growth has started.

3. Seedlings of peanuts whose cotyledons have been removed after six days' growth will form chlorophyll for a longer time than will seedlings of beans similarly treated. The gradual decrease in the intensity of greening is accompanied by a decrease in the amount of yellow pigment.

4. The loss in weight of peanut seedlings with their cotyledons removed is nearly three times that of beans treated in the same way. This is due chiefly to the amount of excess food translocated to the hypocotyls previous to cotyledon removal.

5. The per cent. of shoot length to total length and shoot weight to total weight in starving peanuts was found to be greater than for check plants. This relationship is just the reverse for beans. A very close correlation exists between the per cent. shoot weight to total weight of starving peanuts and beans grown at 23° C.

6. Starving seedlings of peanuts utilize their oil reserve more economically than the checks. After the first six days of inanition they were unable to maintain the regular fluctuations in the per cent. of oil that was shown by the check seedlings.

The author wishes to express his sincere appreciation to Dr. C. F. HOTTES for his suggestions and helpful criticism throughout the course of the work.

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COMPARISON OF THE CHEMICAL COMPOSITION OF SOME MARINE ALGAE

MARGARET R. BUTLER

Very few analyses of marine algae of the Atlantic coast have been reported in the literature as compared with those of the Pacific coast forms. There exist, furthermore, sharp differences in the type and abundance of species in these two regions. For Canadian waters of the Atlantic coast no analyses have yet been reported. The abundance of this material on the shores of the Maritime Provinces establishes it as a natural resource of possible importance. With this idea in mind analyses have been carried out on the more abundant forms for their content of substances of economic importance. In other words, the present report is intended as a survey as well as a comparison with the same or allied species to be found in other parts of the world.

Until displaced in 1873 by the iodides of the great salt deposits, seaweed was the world's chief source of iodine. Yet during the stress of economic conditions imposed by the world war, it was reverted to as a commercial source of iodine in the United States.

The United States Department of Agriculture also became interested in the production of potash from kelps and operated an experimental and demonstrational kelp products plant at Summerland, California, the account of which appears in "Potash, a review, estimate and forecast" (Wiley), and in a series of articles in the "Journal of Industrial and Engineering Chemistry." It is interesting as having been one of the first utilized products of seaweed.

The sale of dulce in certain localities as a human food and as a medicinal lends interest to the determinations of the protein content calculated from the nitrogen present. In connection with the use of seaweeds as cattle food, and fertilizer, nitrogen vies with potassium in importance.

The difference between the additive figures for cellulose (crude fibre), protein and salts (ash), and the total solid content as determined by the moisture, may be taken as polysaccharide. A complete proximal analysis of six species of algae is thus presented in the following experimental section.

Experimental

PREPARATION OF SAMPLES

The samples used in the following analyses were composed of a number of plants collected at the same time from the same place. These data have been noted for each sample. Individual collections have been kept separate

on account of seasonal and ecological variations; a number of plants have been included in order to make the samples as uniform and representative as possible. For identification of species reference has been made to FARLOW's "Marine algae of New England." (7).

To lessen the effect of autolysis and bacterial action, the time elapsing between collection and completion of drying has been cut down to a minimum. In order to approach as nearly as possible to the ideal condition, the plants were kept in sea water until ready for treatment. After removal from the water, as much of the surface moisture as possible was removed by shaking or blotting, and the plants at once ground in a food chopper. To insure that each collection should have the same percentage of surface moisture was an impossibility so that a fairly wide variation in moisture determinations of the same species was foreseen, and table I represents averages.

The advisability of making moisture determinations on minced material might be questioned on the ground that moisture would be pressed out in the grinding. This was found to be true in very few cases, however, and since what little press juice was lost contained considerable solid matter the error introduced was accepted as slight. The chief reason for grinding the plants while fresh and moist is the ease with which it can be accomplished then. Many of them are impossible to grind once they have dried.

The minced material was dried, either in the oven below 100° C. or in the sun. When thoroughly dry it was again put through the chopper until finely powdered. In this form the samples were preserved; and just before using, a portion was dried to constant weight and kept in a desiccator.

TABLE I
MOISTURE CONTENT OF MARINE ALGAE

SPECIES	MOISTURE
	<i>per cent.</i>
<i>Scytosiphon lomentarius</i> (a) ..	91
<i>Laminaria longicruris</i> (a)	89
<i>Porphyra laciniata</i> (c)	86
<i>Rhodomenia palmata</i> (a) and (b)	85
<i>Delessaria sinuosa</i> (a)	83
<i>Chondrus crispus</i> (b)	77
<i>Fucus vesiculosus</i> (a) and (b) . .	76
<i>Ascophyllum nodosum</i> (c)	70
<i>Gigartina mamillata</i> (c)	68

(a) Collected at St. Andrew's, New Brunswick, in June.

(b) Collected at St. Andrew's, New Brunswick, in July.

(c) Collected at Halifax, Nova Scotia, in November.

MOISTURE DETERMINATION

These determinations were made by drying a weighed sample of the minced plant, usually 10–20 grams, to constant weight in the oven below 100° C. The loss of weight was calculated as moisture. Thirty hours were usually sufficient for complete drying. Percentages of moisture in fresh samples examined are presented in table I.

ASH DETERMINATIONS

Moisture-free samples of 3 to 4 grams were used in all cases and ignited in an electric muffle furnace at 575°–600° C. for five hours. In table II will be noted the analyses of the ash of two species hitherto unrecorded. That of *Laminaria longicruris* is remarkably high. My average figure for *Laminaria* of 21.9 per cent. agrees very well with that of REED of 21.1 per cent. For the most part the agreement is good and does not show any marked difference between the salt content of northern Canadian forms and of those of Hawaii (REED) and of France (VINCENT, 26).

TABLE II

COMPARATIVE TABLE OF ASH VALUES AS PERCENTAGE OF DRY WEIGHT

SPECIES	RESULTS OF PRESENT INVESTIGATION	PREVIOUS RESULTS	BY WHOM REPORTED
<i>Laminaria longicruris</i> (a)	33.2	—	HOAGLAND (11)
<i>Laminaria andersonii</i>	—	26.5	
<i>Laminaria</i> (average of several species) (d)	21.9	21.1*	REED (20)
<i>Chondrus crispus</i> (a)	27.7	14.2*	REED (20)
		14.6	HAAS (9)
		24.9	VINCENT (26)
		36.6	VINCENT (26)
<i>Rhodomenia palmata</i> (a)	26.9	—	VINCENT (26)
<i>Gigartina mamillosa</i> (c)	22.5	—	
<i>Fucus vesiculosus</i> (a)	20.7	22.2	VINCENT (26)
<i>Fucus serratus</i>	—	26.3	VINCENT (26)
<i>Ascophyllum nodosum</i> (b)	22.2	23.6	VINCENT (26)
<i>Porphyra laciniata</i> (a)	15.7	10.37*	REED (20)

* Miss REED's figures are for air dry material, not dry weight.

(a) Collected at St. Andrew's, New Brunswick, in July.

(b) Collected at Halifax, Nova Scotia, in November.

(c) Collected at Digby, Nova Scotia, in September.

(d) Collected at Halifax, Nova Scotia, in September.

POTASSIUM DETERMINATION

Potassium determinations were made on the ash residues prepared as previously described, and results calculated to dry weight. The method

used was that of KRAMER and TISDALL (13) for the estimation of potassium in blood serum. A definite weight of each ash, 0.10 to 0.25 gram, was dissolved with the aid of 1 or 2 cc. of sulphuric acid, and the solution made up to 100 cc. These solutions were used to replace the serum of the original method. The results given in table III are the average of duplicate analyses, only one of which varied more than 0.03 cc. in the final titration.

When the figures in table III are compared the conclusion may be drawn that potassium is richer in the specimens which I have analyzed than in those from other water. *Rhodymenia palmata* is a striking instance of this. The only possible exception is that of *Fucus vesiculosus* on the basis of BARLOW's analysis. I have been unable to find any analysis in the literature for the potash content of *Gigartina* or *Porphyra*. The relationship between content of ash and of potassium will be seen in table VII.

TABLE III

COMPARATIVE TABLE OF POTASSIUM VALUES AS PERCENTAGE OF DRY WEIGHT

SPECIES	RESULTS OF PRESENT IN- VESTIGATION	PREVIOUS RESULTS	BY WHOM REPORTED
	<i>per cent.</i>	<i>per cent.</i>	
<i>Rhodymenia palmata</i> (a)	12.22	2.42	WHEELER & HARTWELL (28)
		4.48	VINCENT (26)
<i>Laminaria longicuris</i> (a)	7.33	—	
“ <i>digitata</i>		5.40	HENDRICK (19)
“ “		5.49	WHEELER & HARTWELL (28)
“ <i>andersonii</i>		2.48	BURD (3)
“ <i>saccharina</i>		2.08	WHEELER & HARTWELL (28)
<i>Chondrus crispus</i> (a)	4.58	1.53	WHEELER & HARTWELL (28)
<i>Fucus vesiculosus</i> (a)	3.39	6.29	BARLOW (2)
		2.24	VINCENT (26)
		1.50	WHEELER & HARTWELL (28)
<i>Gigartina mamillosa</i> (b)	3.18	—	
<i>Porphyra laciniata</i> (a)	2.69	—	

(a) Collected at St. Andrew's, New Brunswick, in July.

(b) Collected at Digby, Nova Scotia, in September.

IODINE DETERMINATIONS

Iodine was determined in moisture-free samples by KENDALL's method (12), the amount of sodium hydroxide used for fusion, however, being reduced by half.

There is in the literature a very extensive series of iodine determinations. Moreover in these analyses there is a high percentage of variation, partly due to seasonal variations and partly to technical errors. I report

analyses for several species hitherto undetermined. Table IV reveals many points of close agreement. The Canadian forms do not show any consistently higher level of iodine content. *Laminaria longicuris* is notably low in iodine for its genus.

TABLE IV
COMPARATIVE TABLE OF IODINE VALUES AS PERCENTAGE OF DRY WEIGHT

SPECIES	RESULTS OF PRESENT IN- VESTIGATION	PREVIOUS RESULTS	BY WHOM REPORTED
	<i>per cent.</i>	<i>per cent.</i>	
<i>Rhodomenia palmata</i> (a) & (c)	0.0231	0.122 0.7120 0.081	CAMERON (4) STANFORD (23) VINCENT (26)
<i>Prophyra laciniata</i> (a)	0.0085	—	—
“ <i>vulgaria</i>	—	0.009	CAMERON (4)
<i>Chondrus crispus</i> (b)	0.0736	0.053	CAMERON (4)
<i>Gigartina mamillosa</i> (c)	0.1237	0.016	CAMERON (4)
<i>Ascophyllum nodosum</i> (b)	0.1362	0.0572 0.084	STANFORD (23) VINCENT (26)
<i>Fucus vesiculosus</i> (a)	0.0127	0.0297 0.015 0.028	STANFORD (23) WEIBULL (21) VINCENT (26)
<i>Laminaria longicuris</i> (a)	0.0737	—	—
“ <i>digitata</i> (d)	0.349	0.45 0.5 0.4535 (stipe) 0.2946 (frond)	DOHERTY (6) HENDRICK (10) STANFORD (23) STANFORD (23)
<i>Agarum turneri</i> (d)	0.097	—	—
“ <i>fimbriatum</i>	—	0.09 0.022	TURRENTINE (25) CAMERON (4)
<i>Alaria esculenta</i> (d)	0.101	—	—
“ <i>lanceolata</i>	—	0.06	TURRENTINE (25)
<i>Halosacchion ramentaceum</i> (d)	0.029	—	—
“ <i>glandiforme</i>	—	0.006	CAMERON (4)

(a) Collected at St. Andrew's, New Brunswick, in June.

(b) Collected at Halifax, Nova Scotia, in November.

(c) Collected at Digby, Nova Scotia, in September.

(d) Collected at Halifax, Nova Scotia, in September.

NITROGEN DETERMINATIONS

Percentage of nitrogen in moisture free samples was estimated by Kjeldahl's method, and does not therefore include nitrate nitrogen. The figures obtained for nitrogen are in agreement with those of previous investigators and require no comment in themselves. If it is assumed that this nitrogen comes from protein and is calculated as such the value obtained

represents only a small portion of the organic matter present. This sharply differentiates the metabolism involved.

TABLE V
COMPARATIVE TABLE OF NITROGEN VALUES AS PERCENTAGE OF DRY WEIGHT

SPECIES	RESULTS OF PRESENT IN- VESTIGATIONS	PREVIOUS RESULTS	BY WHOM REPORTED
	<i>per cent.</i>	<i>per cent.</i>	
<i>Rhodymenia palmata</i> (a)	3.71	2.71 1.84	WHEELER & HARTWELL (28) VINCENT (26)
<i>Porphyra laciniata</i> (a)	3.61	5.55*	REED (20)
<i>Gigartina mamillosa</i> (b)	3.54	—	
<i>Laminaria longicruris</i> (a)	1.91	—	
“ <i>digitata</i>	—	1.74 1.23	WHEELER & HARTWELL (28) HENDRICK (10)
“ <i>saccharina</i>	—	1.63	WHEELER & HARTWELL (28)
“ <i>andersonii</i>	—	2.4	HOAGLAND (11)
<i>Laminaria</i> (average of 7 species)	—	2.4	BURD (3)
<i>Chondrus crispus</i> (a)	1.65	0.89* 2.24 2.26	REED (20) VINCENT (26) WHEELER & HARTWELL (28)
<i>Fucus vesiculosus</i> (a)	1.37	1.5 * 2.43 1.61 2.29 0.99	REED (20) VINCENT (26) WHEELER & HARTWELL (28) BARLOW (2) HENDRICK (10)

* REED'S figures are for air dry, not moisture free samples.

(a) Collected at St. Andrew's, New Brunswick, in July.

(b) Collected at Digby, Nova Scotia, in September.

CRUDE FIBER DETERMINATION

Crude fiber was determined according to the method given by LEACH (16) for its estimation in cereals. There have been few determinations of crude fiber in algal forms. The values are fairly constant for the species analyzed and they are consistently low. This absence of the cellulose complex among the red and brown forms can be taken as another characteristic of their metabolism.

Table VI presents a comparison of crude fiber values of a number of species, and in table VII will be found a summary of the constituents of six species found abundantly on the coast of the Maritime Provinces of Canada. By the addition of the values for ash, crude fiber and protein ($N \times 6.25$) the difference may be taken to be carbohydrate other than cellulose. Thus

50-60 per cent. of the total solid content of these algae is in this form which is probably mostly polysaccharide judging from personal experience.

TABLE VI

COMPARATIVE TABLE OF CRUDE FIBRE VALUES AS PERCENTAGE OF DRY WEIGHT

SPECIES	RESULTS OF PRESENT IN- VESTIGATIONS	PREVIOUS RESULTS	BY WHOM REPORTED
	<i>per cent.</i>	<i>per cent.</i>	
<i>Fucus vesiculosus</i> (a)	5.21	—	REED (20) HOAGLAND (11)
<i>Laminaria longicuris</i> (a)	3.61	—	
“ (average of 6 species)	—	6.7*	
“ <i>andersonii</i>	—	10.4	
<i>Porphyra laciniata</i> (a)	2.75	—	REED (20)
<i>Chondrus crispus</i> (a)	2.15	2.2*	
<i>Gigartina mamillosa</i> (b)	2.05	—	
<i>Rhodomenia palmata</i> (a)	1.50	—	

* REED'S figures are for air dry, not moisture free samples.

(a) Collected at St. Andrew's, New Brunswick, in July.

(b) Collected in Digby, Nova Scotia, in September.

TABLE VII

COMPOSITE TABLE

SPECIES	MOIS- TURE*	ASH	POTAS- SIUM	IODINE	CRUDE FIBER	NITRO- GEN	COMMON NAME
	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>	
<i>Laminaria longicuris</i>	88.5	33.2	7.35	0.0737	3.61	1.91	Kelp
<i>Fucus vesiculosus</i>	76.4	20.7	3.39	0.0127	5.21	1.37	Rockweed
<i>Rhodomenia palmata</i>	85.4	26.9	12.22	0.0231	1.50	3.71	Dulse
<i>Porphyra laciniata</i>	85.7	15.7	2.69	0.0085	2.75	3.61	—
<i>Chondrus crispus</i>	76.8	27.7	4.58	0.0736	2.16	1.65	Irish moss
<i>Gigartina mamillosa</i>	78.0	22.5	3.18	0.1237	2.05	3.54	—

* All analyses except that of moisture are expressed as percentage of dry weight.

Discussion

The influence of ecological factors on the composition of marine algae must first be stressed; and this is a fact which, unfortunately, has not been appreciated adequately by previous investigators. Habitat and season of collection age of plant (8), temperature and salinity of surrounding water,

and depth of submergence, all have an influence on the nature and amount of constituents present in the plant. For example, SAUVAGEAU (22) compared the efflorescence on two specimens of *Rhodymenia palmata*; one was collected on the coast of France, the other on the coast of Iceland. On the plant from Iceland he found both potassium chloride and mannitol; on the plant collected at Roscoff, potassium chloride but no mannitol. LAPICQUE (15) found *Laminaria flexicaulis* to contain two per cent. soluble carbohydrates in the early spring as compared with eighty-one per cent. in the late summer. Mineral salts also vary with the seasons. Furthermore, the percentage of the same element, e.g. potassium or iodine, varies in different parts of the same plant. In making comparisons of analyses of the same species as well as of different species these facts must be constantly considered. For the reason that there is a large unavoidable error introduced in the determination of the moisture content of algae, it is highly desirable that analyses of other constituents be expressed in terms of the dry weight.

As might be expected the species having the highest ash values are also those with the highest content of potassium. From table VII it is apparent that potassium constitutes a considerable portion of the ash. This is especially true for *Laminaria longicruris* and *Rhodymenia palmata*.

The values for iodine are difficult to compare because of the readiness with which it is lost in preparatory handling. It will diffuse readily from the dead plant, (DANGEARD, 5) and is volatile in the free form, (SAUVAGEAU, 22). The apparent presence of iodine in the free form, as iodide, and in organic combination, in varying degrees (KYLIN, 14) makes the problem a difficult one. The volatility of potassium iodide at high temperatures introduces a possible technical error.

The use of algae and their products as food, though inconsiderable on the North American continent, is extensive in other parts of the world. Their value as such is dependent on the mineral salts and proteins which may be present. SAIKI (21) has shown that the carbohydrates present are very difficult to hydrolyze by means of enzymes, and hence are almost indigestible to man; and only slightly digested even by ruminants. Regarding the proteins of algae little is known and much work needed. HOAGLAND (11) gives nitrogen values from 0.98 to 2.67 per cent. dry weight of the Pacific Coast kelps, of which 13 to 37 per cent. is in non-protein form. Therefore the maximum calculated on this basis would be 14.5 per cent. protein. In the present investigation a maximum nitrogen value of 3.71 per cent. dry weight, (exclusive of nitrates), was found in *Rhodymenia palmata*, which, converted by the factor 6.25, gives 23.19 per cent. protein. KYLIN (14) reports that ammonium nitrate occurs in all, nitrates in some, but not in others.

STEWART (24) testing the efficacy of algae as a fertilizer, found the nitrogen present to be readily available in some species, less readily in

others. The fact that 50 per cent. of the dry weight of organic matter is capable of furnishing humus to the soil (3) is an added advantage in a fertilizer. Potassium, which is essential to a good fertilizer, is present chiefly as chloride and sulphate (1).

It has been difficult to find similar analyses reported for the same species with which the present work was concerned. This is due to the fact that they are not indigenous to the Pacific Coast where any work done on this continent has been carried on. Where it was found impossible to compare with the same species, other species of the same genus have been included, if available. On the comparative tables relative to each determination, quotations have been given from a number of workers, in order to emphasize the fact that there is such a wide variation in the results, which might be considerably lessened if all the limiting factors were known.

Summary

Several species of algae common to the North Atlantic Coast have been analyzed for their content of moisture, ash, potassium, iodine, protein and crude fiber.

Moisture determinations on nine species were found to average 80 per cent., with a maximum of 91 per cent., and a minimum of 68 per cent.

Ash determinations averaged 25 per cent. of the dry weight; maximum in *L. longicuris* of 33.2 per cent., minimum in *P. laciniata* of 15.7 per cent.

Potassium determinations on six species gave an average content of 5.57 per cent. of the dry weight; maximum in *R. palmata* of 12.22 per cent., minimum in *P. laciniata* of 2.69 per cent.

Iodine has been determined in twelve species the average content of which is less than 0.1 per cent. of the dry weight. The maximum of 0.349 per cent. was found in *L. digitata*, a minimum of 0.0085 per cent. in *P. laciniata*.

Nitrogen determinations were made on six species, three of which were much higher than the others, and calculated as protein ($N \times 6.25$) would average 22.6 per cent.

Crude fiber determinations were made on six species and only one found to be above 5 per cent.; the majority are less than half of this amount.

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RELATION OF PHOTOTROPISM TO THE WAVE-LENGTH OF LIGHT*

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(WITH TWO FIGURES)

Many conflicting theories and statements appear in plant physiological literature regarding phototropism. Such conditions frequently exist in a young science where much of the earlier work is qualitative. In many of these early experiments dealing with the bending of plant stems toward light, the lights used were such that it was impossible to determine accurately whether the color or the intensity was the predominating cause of the bending. The relative influence of the different colors, or wave-lengths, could not be determined because different intensities were used. Both of these light factors must be considered, as a greater value of one may off-set the lesser value of the other. In order to determine which colors are most effective in phototropic bending, it is necessary to use wave-lengths of equal intensities.

One method of evaluating the wave-length effects is described by HURD.¹ Wratten light filters were used between the light source and the fucus spores and rhizoids with which this work was performed. The intensities were made equal by increasing or decreasing the distance of the electric arc from the measuring instrument. PARR,² working with *Pilobolus* under carefully controlled conditions, found that light in all regions of the visible spectrum brought about responses in this plant. The presentation time decreased gradually from red to violet, with no indication of intermediate maxima or minima. This work appears to be at variance with statements appearing even in recent text books on plant physiology, that the more highly refrangible rays of light are most effective in phototropic movement with the effect diminishing from blue to yellow, and again increasing in the red and infra-red.

PRIESTLEY³ has recently attempted to give a rational explanation to the phenomenon of phototropism. He shows that phototropic curvature in coleoptiles is consistent with the "light-growth" hypothesis, in spite of

* The investigations herein described have been carried out in cooperation with the University of Maryland.

¹ HURD, ANNIE MAY. Some orienting effects of monochromatic lights of equal intensities on fucus spores and rhizoids. *Proc. Nat. Acad. Sci.* 5: 203-204. 1919.

² PARR, ROSALIE. The response of *Pilobolus* to light. *Ann. Bot.* 32: 177-205. 1918.

³ PRIESTLEY, J. H. Light and growth. *New Phyt.* 24: 271-283. 1925, and 25: 145-170; 213-247. 1926.

many seemingly discrepant experiments. The amount of light required to induce phototropic curvature in normal light-grown shoots is greater, and must be continued longer, than that required to bring about similar curvatures in *etiolated* shoots. It is thus evident that light affects normal and etiolated shoots quite differently. Briefly, the mechanism of bending in etiolated shoots as discussed by PRIESTLEY, is as follows: The walls of the cells making up the tissue contain fat and protein. These substances prevent the ready passage of sap and water from the vascular system to the meristematic tissue which, under favorable conditions, is capable of rapid growth. BLAAUW points out the similarity between light and its photochemical effect on a photographic plate on the one hand and an etiolated coleoptile on the other. Relatively small quantities of light produce photochemical actions on these shoots. Protein and fatty materials disappear from the cell walls, with the latter substances migrating mainly to the cuticle. The passage way between the meristematic cells and their water and food supply is opened up. In the words of PRIESTLEY, "Increased surface growth now ensues. Growth as a whole may be as active as ever on the more brightly lit side of the etiolated shoot, but it is differently distributed. More cells are added to the surface of the stem and leaf, and less proportionately contributed to the inner layers of the shoot axis. The result is, therefore, in the aggregate, a retardation of growth in length on the illuminated side, and a positive phototropic curvature."

Two classes of explanations for the modification in growth rate have been proposed: First, those assuming the effect to be predominantly due to a local temperature change brought about by unsymmetrical absorption of radiant energy; second, those postulating some type of photochemical action. In this second group would fall both those theories involving a local and direct photochemical change at the point of absorption of light, and those assuming a type of hormone action. The first group, involving a purely thermal hypothesis, would lead to a prediction of phototropic bending for all regions of the spectrum where the radiant energy could be absorbed by the cell materials. Infra-red for all wave-lengths longer than 1.1μ is absorbed by the water, even in relatively small layers. The remainder of the cell materials would absorb light pretty generally through the entire visible spectrum. Where etiolated plants are used, with the absence of chlorophyll, a very selective action would not be expected. In the case of a photochemical hypothesis, however, one might have a highly selective type of absorption, either showing maxima and minima, or beginning weakly at some particular wave-length and increasing towards the shorter wave-lengths or blue end of the spectrum. Such selective absorption in the visible or ultra-violet is characteristic of electronic changes in energy within the molecule.

In the present experiment it was proposed to determine whether or not selective regional phototropic responses (*i.e.*, to different colors) would be found for equal intensities. Second, to determine, in case of selective responses whether the effect of different colors could be off-set by modification in intensities. Such an experiment would crucially decide between the two classes of hypotheses.

The general underlying idea of determining the relative influence of colors in a differential manner by exposing the plant to two different colors from opposite sides was proposed earlier by JOHNSTON.⁴ The present experiment was simply an elaboration of this proposed method, wherein particular attention has been given to the physical quantities involved. The wave-length regions have been restricted by filters whose transmission curves were definitely determined. The intensities were measured by means of an especially constructed thermocouple and galvanometer system of high sensitivity, with which accurate determinations could be made over an unusually large range of relative intensities.

The details of the apparatus are as follows: The thermocouple-galvanometer system constitutes essentially a blackened thermometer whose rise in temperature above the surrounding room temperature is proportional to the amount of radiation falling upon it, and is practically independent of the wave-length of the radiation. The rise in temperature was indicated by a galvanometer deflection which was read by the displacement of a small band of light along a metric scale. The system consisted of a single closed electrical circuit of which the d'Arsonval galvanometer coil was a part. Where dissimilar metals are used in a circuit, the unequal heating of the points of contact causes an electromotive force. For small differences in temperature this electromotive force, and the current arising, is proportional to the difference in temperature.

The galvanometer coil and most of the circuit were constructed of copper. The remainder of the circuit was made up of a short length of fine bismuth wire and a short length of bismuth-tin alloy. The juncture of the copper with the bismuth, and the juncture of the copper with the bismuth-tin were each maintained at room temperature. To the point where the bismuth joins the bismuth-tin wire, a blackened receiver was attached. Radiant energy falling upon such a receiver raises its temperature above that of the junctures to the copper, and so causes the current. The alloy of bismuth-tin was made up of 95 per cent. bismuth and 5 per cent. tin. It is interesting to note that this small percentage of tin yields a material of opposite thermoelectric characteristics to pure bismuth.

In the choice of materials for construction of such a thermocouple, not

⁴ JOHNSTON, EARL S. A plant photometer. *Plant Physiol.* 1: 89-90. 1926.

only the thermoelectric power, but also the resistance and the thermal conductivity should be considered. This combination of metals is the best known at the present time. The wire used was about 25 microns in diameter and between 2 and 3 mm. long. The receiver was a circular piece of platinum foil about 2 mm. in diameter. The thermocouple was then placed in a vacuum of better than 10^{-4} mm. pressure thereby serving a double purpose. First, the sensitivity was increased by the removal of convection loss, and second, small disturbances which ordinarily would reach the thermocouple through convection were eliminated. When radiant energy is focused upon such a receiver by means of a lens, it is equivalent to greatly increasing its area, and thus yielding a greater response than an actual increase in area would give, because the thermal losses would be increased at the same time. It is necessary, however, that the same effective area be used throughout the experiment. Because of the very great range of intensity to be observed, it was necessary to use a number of resistances in series and parallel, in order to change the sensitivity by several known factors.

The plant photometer box was 238 cm. in length and 30.5 cm. wide. It was divided into five compartments instead of three as in the earlier experiment. The end, or lamp compartments, were 59 cm. long and 59 cm. high, while the other three were 44 cm. high. The central, or plant compartment (87 cm. in length), was insulated from each lamp compartment by a filter chamber 10 cm. wide. Before the light could reach the plant from either side, it was passed through a water filter immediately surrounding the lamp, through a plate glass window, then through the filter chamber which was cooled by a stream of air, and finally through the desired color filter and a water cell, one window of which was made of heat absorbing glass. This water cell was set at an angle to avoid reflecting light to the plant from the opposite side of the plant chamber.

By the introduction of the cooled filter compartments between the lamp compartments and the plant chamber, heat necessarily arising in the lamp chambers was prevented from reaching the plant compartment. By means of the water cell with the heat absorbing window on the lamp side, only visible radiation was allowed to reach the plant. Water absorbs all the radiation in the infra-red longer than $1.1\ \mu$, while the heat absorbing filter cuts out the near infra-red region between $1.1\ \mu$ and the visible. In the plant chamber the seedling was surrounded by a double-walled glass cylinder, the space between the walls being filled with water and the entire cylinder slowly rotated around the plant axis in order to equalize temperature conditions in the immediate environment of the seedling. The interior walls of all five chambers were painted a dull black. A general view of the photometer box is shown in figure 1.

The coleoptile of the oat was selected for these experiments. The seeds were germinated between glass plates covered with wet filter paper. The plates were so arranged in moisture chambers that the seedlings grew vertically. The seedlings were carefully selected for straightness and transferred to small Erlenmeyer flasks fitted with cork stoppers. Each seedling was supported by means of a little cotton in a small hole of the stopper. The flask was filled with distilled water so that the roots were entirely immersed. By means of a cross hair in a telescope the seedling was adjusted to a vertical position in the plant compartment between the light filters.



FIG. 1. General view of plant photometer with door of plant chamber removed to show double-walled glass cylinder in which seedling was grown.

In conjunction with the heat absorbing cell, four different light filters (2" x 2") were used in these preliminary experiments. Three were Wratten filters numbers 24 (red), 61 (green), 47 (blue) made by the Eastman Kodak Company, and one a "heat resisting yellow" (yellow shade) made by the Corning Glass Company.

The curves presented in figure 2 indicate the transmission of these filters. From these curves it will be observed that the red filter transmits freely all wave-lengths greater than 6000 Å. and absorbs all wave-lengths shorter than 5800 Å., in fact, cutting off practically all

wave-lengths shorter than 5900. The yellow filter transmits freely all wave-lengths longer than 5400 and cuts off all wave-lengths shorter than 5200, effectively removing all wave-lengths shorter than 5350 Å. The green filter shows a maximum transmission at about 5100, practically cutting off all wave-lengths beyond 6000 on the red side, and 4800 on the blue side, with no measurable transmission shorter than 4700. The blue filter transmits effectively a region from 3900 to 5000.

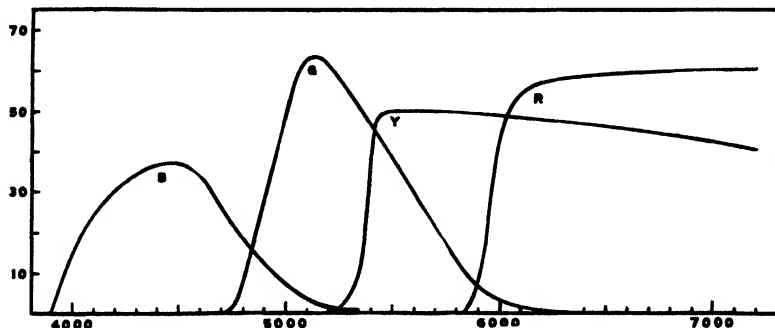


FIG. 2. Curves representing percentages (ordinate) of transmission of the light filters; blue (B), green (G), yellow (Y), and red (R). Wave-lengths are expressed in Angström units along the abscissa.

The results obtained were as follows. In the first place where the plant was exposed to radiation from only one side with the water cells all removed, and a filter introduced which absorbed all the visible light, no phototropic bending could be observed. In other words, infra-red radiation between the visible and $2.5\ \mu$ produced no phototropic bending which could be observed. Second, with the heat absorbing filters together with the red filter and no light from the opposite side, no measurable phototropic bending could be detected. Hence no wave-lengths longer than 6000 Å. could be found to produce a measurable phototropic effect. Similarly, with the yellow filter substituted for the red filter, the plants were grown with no opposing light. In this case, a noticeable bending was soon apparent. Hence a phototropic influence is certainly to be found in the region between 5200 and 6000 Å units, and probably in the narrow region between 5350 and 5900 Å. This is the region usually termed yellow. It is impossible from the present experiment to say which portion of this region may be effective. For the green and blue filters the unbalanced phototropic effect was very marked, the bending taking place in a very few minutes. The first differential balance was observed with the yellow light on one side and the green light on the other. A balance was actually secured, however, only when the yellow light was 1,000 times more intense than the green light as determined by the non-selective thermocouple-galvanometer mea-

surements. In the same way a balance was reached between the green and blue when the green light was 30 times more intense than the blue.

In each experiment a single seedling was used. The general procedure was to place the seedling between two different light sources and, after a time interval, observe the coleoptile through the telescope in order to determine any growth curvatures. If, for example, the seedling was exposed between the blue and green filters and a distinct bending toward the blue was shown at the end of half an hour, the lights were so adjusted by position and current controlled by rheostats, that the green intensity was increased and the blue decreased. Another seedling was then placed in the photometer and the experiment repeated. After several trials a balance point was reached where the effect of one light was neutralized by the effect of the other, and the plant continued to grow in a vertical position even after an exposure of several hours. When this balance point was reached the seedling was removed and the especially constructed thermocouple placed in the position of the plant. The relative intensities of the lights were then measured by means of the galvanometer.

It is of interest to note that repetition of the experiment yielded balance points differing by less than 5 per cent. from the previous experiment. Such reproducibility is somewhat unusual in biological measurements. It suggests that by this method one is observing a characteristic of some underlying photochemical reaction. The absence of effect in the red and infra-red together with a very sharp increase in passing from yellow to green, and the subsequent rise in the blue, is typical of an electronic photochemical reaction. The experiment must be regarded, therefore, as crucial evidence against a purely thermal theory and strong support for a photochemical theory.

The results seem of sufficient importance to justify a more elaborate experiment wherein narrower spectral regions are used, that is, more restricted color ranges, thus enabling one to determine the phototropic effect at many points through the spectrum. For this purpose a monochrometer must necessarily be used. In such an experiment it seems desirable also that the phototropic influence of all the wave-length regions be determined in terms of a single comparison band. The intensity of the comparison light could be varied to counterbalance successively the phototropic influence of each band or color, taken preferably all at the same intensity. The experiment should also be carried into the ultra-violet in order to gain the additional information which may throw some light upon the nature of the photochemical reactions involved.

VARIATIONS IN THE O_2 OF PLANT TISSUES

WILLIAM A. BECK

(WITH FOUR FIGURES)

The physical chemists have expressed widely different views regarding the nature of osmotic pressure, but while advancing different theories in explanation of the phenomenon, they agree in the quantitative expression of the so-called pressure. If the osmometer containing the solution in question be placed into the solvent, the difference of pressure on the solution and the solvent expresses numerically the osmotic pressure of the solution in the osmometer, when a condition of equilibrium exists, *i.e.*, when no solvent flows in either direction, from or to the solution through the membrane.

The definition of osmotic pressure is illustrated in figure 1. If the

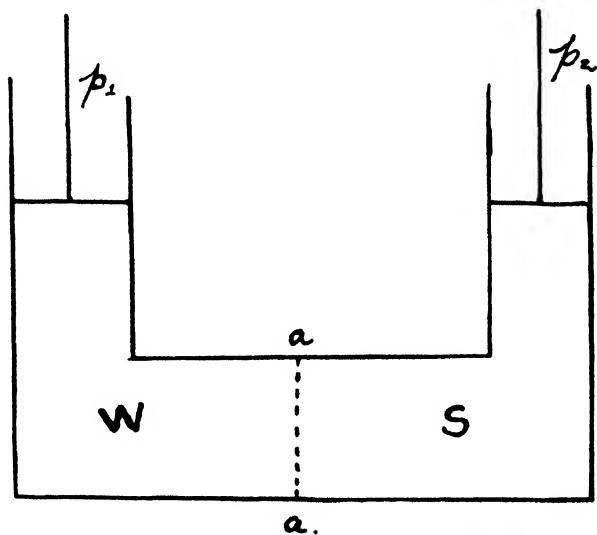


FIG. 1. A diagrammatic illustration of osmotic pressure. W is the solvent, S is the solution, a is the membrane; p_2 is the pressure on the solution, p_1 is the pressure on the solvent. In a condition of equilibrium $p_2 - p_1$ is the numerical expression of the osmotic pressure.

pressure on the solution (p_2) is too small solvent (W) will flow through the membrane (a) into the solution (S). If it is too great, solvent will flow from the solution (S) into the solvent (W), passing through the membrane in the reverse direction. If the solvent fails to flow in either direction a condition of equilibrium exists at the membrane; under this condition, the difference of the mechanical pressures p_2 and p_1 expresses the osmotic pressure of the solution (S).

$$P = (p_2 - p_1)$$

There is nothing ideal about this quantity, it is very real; it is expressed in atmospheres.

This quantity is not under discussion in this paper and is mentioned here only to emphasize the fact, because some confusion exists about the terms in current literature on this subject.

It is practically impossible to measure the osmotic pressure of the normal cell sap directly. Some investigators measure the osmotic pressure indirectly of sap expressed from plant tissues, by the indirect cryoscopic method. This method can hardly yield results that will be helpful in determining physiological activities in plant tissues. I think this will appear from the results which are presented in this paper.

When a cell is placed into a solution of sufficient concentration, plasmolysis occurs (fig. 2). The receding film of plasm can readily be de-

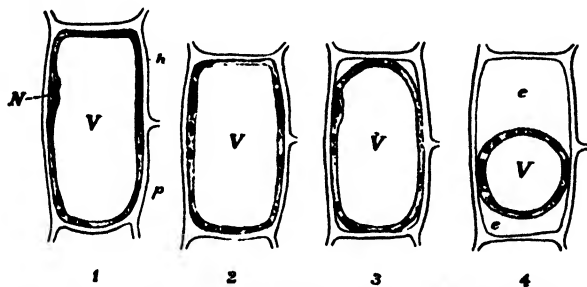


FIG. 2. Various stages of plasmolysis (After DeVRIES).

tected even at incipient plasmolysis, if the illuminating conditions are favorable. If the incipient stage of plasmolysis remains constant, the relative concentration of the plasmolysing agent is taken as a measure of the relative concentration of the sap within the cell at that stage. The concentration of the agent is expressed in gram molecular units (volumetric system). This quantity which expresses the relative concentration of the cell sap at incipient plasmolysis, is termed the *osmotic value* of the cell, at incipient plasmolysis; it is symbolized by O_g . This quantity should always be expressed in mols and not in atmospheres. If the equivalent atmospheres are to be expressed for any reason, evidently only such an agent should be employed for which the equivalent atmospheres of osmotic pressure have been accurately determined. For various reasons given elsewhere, cane sugar was employed as the plasmolysing agent in these studies, and when it became necessary for clearness, the formula for cane sugar was appended to the symbol for the osmotic value.

No attempt is made in this paper to draw conclusions about the relative concentration of the cell sap in the normal condition of the cell. The

object is to study variations in the value of O_k . When there is a variation, it indicates a variation in the *quantity* or the *nature* of the solutes in the cell sap, or a variation in both the nature and the quantity of the solutes.

Some investigators tried to show that there is an O_k gradient in the direction of the flow of water in the plant. URSPRUNG and BLUM showed that there is a gradient in the quantity which expresses the potential ability of the cell to absorb water (they call it suction force), and that the O_k varies irregularly. Without data on the osmotic values of the cell sap and the relative volumes of the cell in the normal condition at incipient plasmolysis and when saturated, no conclusions can be drawn about the potential ability of the cell to draw water. From this it is clear that the problems taken up here are quite different from the problems which URSPRUNG and BLUM discuss under the name of Suction Force studies.

The cells of a given tissue do not as a rule have exactly the same O_k . When fifty per cent. of the cells of a tissue are slightly plasmolysed, the concentration of the agent is taken as the average value; it is expressed as the O_k of the tissue. Numerous tests have shown that this value is reliable, *i.e.*, different tests made at short intervals under the same conditions on a given tissue, yield the same results.

Different tissues in a given plant have widely different O_k 's. This will be clearly shown in the data that follow. Within limits the O_k of a given tissue is characteristic for that tissue. Assimilating tissues for example have higher values as a rule, than epidermal tissues. The range of variation of O_k in response to given factors, is not as great in the epidermal tissues as it is in the assimilating tissues. The O_k of the guard cells varies rapidly within wide limits, under the influence of certain external factors. An example by way of illustration: During the months of March and April, a considerable number of O_k measurements were made for the tissues of ivy leaves all of which were taken from the same plant. The highest value recorded for the epidermis was 0.775; the lowest 0.6; the values found most frequently was 0.65. The highest value recorded for the palisade parenchyma was 1.25, the lowest 1.05, and 1.1 the most frequent value. For the guard cells the lowest was 0.6, the highest 0.825 and the most frequent 0.65.

In table I the O_k is recorded for the lower epidermis, the guard cells and the spongy parenchyma of twenty different plants. The O_k seems to depend upon the nature of the plant as well as upon the environment in which the plant grows. The table has been arranged in such a way as to indicate the relation of the O_k to the nature of the plant. Not more than ten minutes intervened between the measurements of the O_k for the different tissues of a given plant. For any given plant the epidermal tissue invariably showed a lower value than the spongy parenchyma; the guard cells had nearly the

TABLE I
THE O_2 ($C_{12}H_{22}O_{11}$) OF THE TISSUES OF LEAVES

HERBACEOUS				WOODY			
PLANT	LOWER EPIDERMIS	GUARD CELLS	SPONGY PAREN- CHYMA	PLANT	LOWER EPIDERMIS	GUARD CELLS	SPONGY PAREN- CHYMA
<i>Solanum nigrum</i>	0.425	0.675	0.575	<i>Ligustrum vulgare</i>	0.65	0.75	0.975
<i>Crambe maritima</i>	0.375	0.65	0.65	<i>Hybiscus syriacus</i>	0.6	0.55	0.8
<i>Hesperis matronalis</i>	0.55	0.6	0.725	<i>Fraxinus excelsior</i>	0.775	0.825	1.1
<i>Euphorbia lathyris</i>	0.25	0.275	0.575	<i>Cytisus laburnum</i>	0.675	0.9	1.075
<i>Osmunda regalis</i>	0.475	0.425	0.875	<i>Robinia pseudacacia</i>	0.525	0.7	0.9
<i>Polygonum orientale</i>	0.375	0.55	0.675	<i>Liriodendron tulipifera</i>	0.6	0.675	0.825
<i>Musa sinensis</i>	0.4	0.35	0.45	<i>Hedera helix</i>	0.55	0.575	0.675
<i>Datura stramonium</i>	0.3	0.425	0.55	<i>Crataegus oxyacantha</i>	0.55	0.575	0.85
<i>Acanthus spinosus</i>	0.475	0.525	0.625	<i>Castanea sativa</i>	0.575	0.625	0.775
<i>Dioscorea batatas</i>	0.35	0.375	0.6	<i>Populus nigra</i>	0.5	0.575	0.625

same value as the epidermal tissue in some cases, and widely different values in other cases. For example in *Dioscorea batatas* the value was 0.35 for the epidermis, 0.375 for the guard cells and 0.6 for the spongy parenchyma; in *Solanum nigrum* the value was 0.425 for the epidermis, 0.675 for the guard cells and 0.575 for the spongy parenchyma; among the woody plants *Hedera helix* and *Cytisus laburnum* illustrate the same point. The measurements were made on different days but always in the morning between 6:30 and 8:30. During the early hours of the day the influence of the heat and light was not yet as great as it would have been later in the day. The difference of the mean values for the herbaceous plants was 0.0875 between the guard cells and the epidermis, in favor of the guard cells; it was 0.2325 between the spongy parenchyma and the epidermis, in favor of the spongy parenchyma. The difference of the mean values for the woody plants was 0.075 between the guard cells and the epidermis, in favor of the guard cells, it was 0.261 between the spongy parenchyma and the epidermis, in favor of the spongy parenchyma. It is interesting to note that while the actual values are considerably higher in the woody plants than they are in the herbaceous, the differences of the mean values are about the same in the woody and the herbaceous plants. The average value for the epidermis was 0.3975 in the herbaceous plants and 0.6 for the woody; for the guard cells it was 0.485 in the herbaceous and 0.675 in the woody; for the spongy parenchyma it was 0.63 in the herbaceous, and 0.861 in the woody plants.

The results of the experiments, which were carried out to demonstrate the variation of the guard cells in response to the natural factors which influence the plants during the day, are recorded in table II. The values recorded for the guard cells were usually not much different from the value recorded for the epidermis of the same plant, because the readings were taken at an early hour, when the factors had not effected a great variation in the relatively short time of exposure. The measurements recorded

TABLE II

VARIATIONS OF O_2 IN THE GUARD CELLS IN RESPONSE TO LIGHT

PLANT	No.	LOWER EPIDERMIS		GUARD CELLS		SPONGY PARENCHYMA		SHADED
		LIGHT	SHADE	LIGHT	SHADE	LIGHT	SHADE	
<i>Sedum spurium</i>	1	0.275	0.275	0.500	0.4	0.450	0.550	Naturally
<i>Crambe maritima</i>	2	0.375	0.375	0.950	0.625	0.550	0.550	By umbrella
<i>Crambe maritima</i>	3	0.375	0.375	0.950	0.500	0.550	0.550	By foil
<i>Nymphaea alba</i>	4	0.300	0.300	0.550	0.550	0.500	0.500	Naturally
<i>Viola major</i>	5	0.75	0.75	1.250	0.950	0.750	0.750	By foil
<i>Viola minor</i>	6	0.65	0.65	1.300	0.950	0.750	0.750	By foil

in table II were made in the evening. Some plants were exposed to the light during the day, while neighboring plants were shaded. Except in one case, the shaded plants showed considerably lower O_g in the guard cells than did the plants normally exposed to the sun. It is not surprising that the one exception (*Nymphaea alba*) behaved as it did, if the guard cells are understood to be a regulating tissue: The water supply is maximum at all times so that no high degree of regulation is necessary for the rate of transpiration. Plant no. 3, *Crambe maritima* showed the greatest variation, the plant in the shade showing a value of 0.45 mol less than the plant in the light.

A great many measurements that were made on the tissues of leaves, taken from various plants at different times of the same day, tend to show that the O_g of the epidermis does not vary much, if at all, during the day, no matter if the weather be fair or rainy; the guard cells vary considerably, particularly when the weather is fair; the spongy parenchyma varies more than the epidermis, though usually not as much as the guard cells.

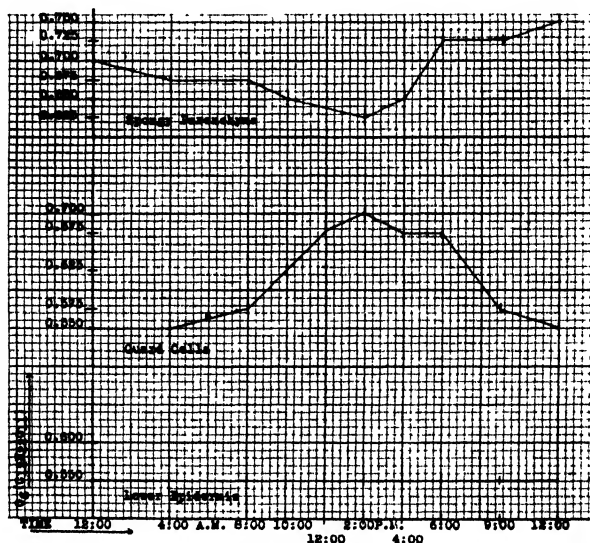


FIG. 3. Diurnal variations of O_g in the tissues of an ivy leaf.

In figure 3 the graphs of the results obtained on the leaf of *Hedera helix* are given by way of illustration of the variations which occur in the epidermis, the guard cells and the spongy parenchyma, during a period of 24 hours.

The value of the epidermis remained constantly at 0.550. The guard cells had an initial value of 0.550 at midnight and at 4:00 A. M.; then an increment set in, which continued at different rates up to 2:00 P. M., at

which time the maximum value was 0.700; the greatest rate of increment was between 8:00 A. M. and noon; the rate was less from 4:00 A. M. to 8:00 A. M. than it was from noon to 2:00 P. M.; the drop from the maximum value after 2:00 P. M. was decided and sharp until 4:00, when no variation occurred until 6:00 P. M., then the decrement was sharper than after 2:00 P. M.; a slower rate of decrement set in at 9:00 and continued until midnight when the initial value of 0.550 was reached.

The variations of the spongy parenchyma were quite different from those of the guard cells. The sense of the variation was usually opposite, and the rates were different. The initial value at midnight was 0.70; there was a decrement up to 4:00 A. M. and then the value remained constant up to 8:00 A. M. (at 0.675). From that time up to 2:00 P. M. there was a decrement with a greater rate of change from 8:00 A. M. to 10:00 A. M. than from 10:00 A. M. to 2:00 P. M. The lowest value at 2:00 o'clock was 0.625. From that time a considerable increment occurred until 6:00 P. M., when the value 0.725 was reached. The value remained the same until 9:00 P. M., when a further increment was experienced up to midnight; then the maximum was reached at 0.750, which was 0.05 mol higher than the initial value.

The facts that it had rained shortly before this experiment was begun, and that during the time of the experiment the barometer was high with sunshine during the day, might be helpful in interpreting these interesting results.

Other plants were examined and found to respond in the same sense, but not always in the same degree. Among these I wish to mention, *Vinca minor*, *Vinca major*, *Cydonia japonica*, *Evonymus japonica*, *Acer negundo*, *Musa sinensis*, *Canna*, *Datura stramonium*, *Nasturtium officinalis*, *Solidago canadensis*, *Taraxacum officinale*, *Plantago major*, *Paeonia officinalis*, *Caltha palustris*, *Sedum telephium*, *Sedum spurium*, *Sempervivum tectorum*, *Crambe maritima*, *Euphorbia lathyrus*, *Sinapis alba*, and *Cobaea scandens*.

As might be expected, the degree of variation was not the same in all of these plants. In *Taraxacum* for instance, the epidermis showed a slight decrement from 7:00 A. M. to 3:00 P. M. (from 0.55 to 0.525). The guard cells manifested a greater increment in *Taraxacum* than in the case of the ivy leaf just cited. At 7:00 A. M. the value was 0.55 and at 3:00 P. M. it was 0.775. The spongy parenchyma decreased its O_2 from 0.8 at 7:00 A. M. to 0.7 at 3:00 P. M. In *Plantago major* the maximum for the guard cells was 1.05 at 2:30 P. M. and the minimum 0.75 at 6:00 A. M. Simultaneously the spongy parenchyma showed a maximum of 0.9 when the guard cells were at minimum, and a minimum of 0.8 when the guard cells were at maximum. *Saponaria ocimoides* failed to show a variation in the spongy parenchyma, as well as in the epidermis, even though the

weather was sunny. The guard cells varied as usual but the increment was 0.1, from 0.65 to 0.75.

Time does not permit further discussion of this interesting subject. I feel convinced that the facts adduced indicate that interesting variations of O_g occur in the plant under the influence of the factors of the natural environment, and that they deserve investigation along the lines suggested.

Annual variations as well as daily variations occur. The results obtained tend to show that some factors which failed to affect a tissue in a short time will eventually affect it after a longer time of exposure. This is particularly true for the epidermis. In figure 4 the graphs of results obtained on an ivy leaf are given.

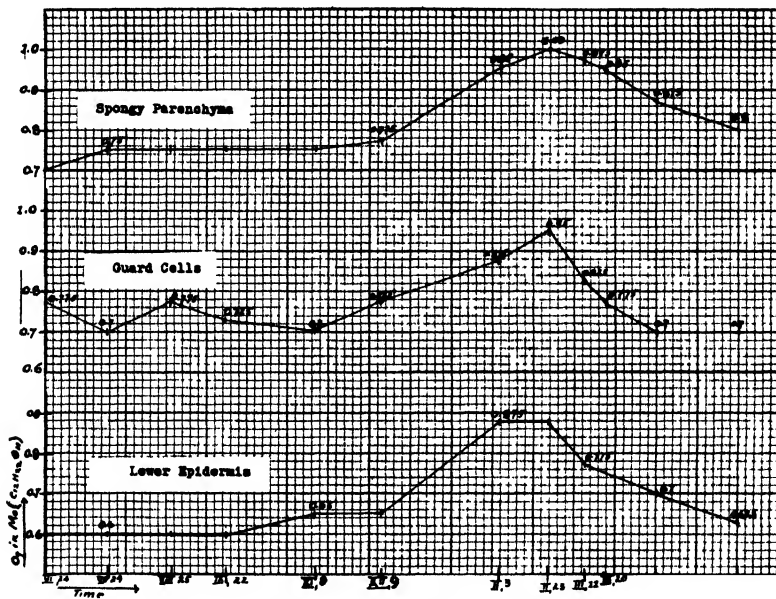


FIG. 4. Annual variation of O_g in an ivy leaf.

On June 24th the O_g of the epidermis was 0.6 mol, and on February 3rd of the following year it was 0.875 mol. The measurements recorded in the graph were made on the same plant which stood in garden soil, exposed to the weather for many years. All of the measurements were made at about 2:00 P. M. The guard cells were the least regular. This might be expected since they respond quickly to certain factors that might have influenced them for a short time, previous to the measurement. The variations in the spongy parenchyma are about as regular as those of the epidermis. It is particularly interesting that all of the tissues showed maximum values on February 23rd. The epidermis reached the maximum

of 0.875 mol on February 3rd already. The other two tissues continued to increase in O_g . The guard cells reached maximum value at 0.95, and the spongy parenchyma at 1.00 mol. The difference between maxima and minima were 0.275 for the epidermis 0.25 for the guard cells, and 0.3 for the spongy parenchyma. It is interesting that these differences are almost the same.

An explanation of these phenomena cannot be offered at present, and the facts are merely recorded for the consideration of other investigators. At some later time when the study of the different influences of certain factors on the O_g of plant tissues has been completed, it is hoped that an adequate explanation may be offered. It would be very helpful in this work to know more of the effect which these factors have on the nature and quantity of the solutes of the sap.

Summary

The O_g ($C_{12}H_{22}O_{11}$) is the osmotic value at incipient plasmolysis, when cane sugar is employed as the plasmolysing agent. It is expressed in molal concentration units. This quantity must not be confounded with the osmotic pressure, nor with the concentration of the cell sap.

Variations in the O_g for a given tissue can serve as an indicator of physiological activities in that tissue. Variations were studied in the epidermis, the guard cells, and the spongy parenchyma.

The O_g is characteristic for a tissue. It is greater in woody plants than in herbaceous plants.

The guard cells respond quickly and considerably to the influence of certain factors in the natural environment. The response in the spongy parenchyma was evident but usually not as rapid as in the guard cells. The response in the epidermis was usually negligible or slow.

The diurnal variation was negligible in the epidermis, considerable in the other two tissues. The character of the variation was different in the spongy parenchyma and the guard cells; at times the increments were opposed in direction.

The annual variations were evident in all three tissues, the O_g increased in all of them from the latter part of Spring to the end of February, when a rapid decrement set in, which continued up to Spring. The increment was least regular in the guard cells. The rate of increment was greatest in all of the tissues during the months of December and January.

FACTORS MODIFYING THE TOXICITY OF PHENOL

WALTER S. EISENMENGER

(WITH ONE FIGURE)

Introduction

That various solids when added to toxic solutions have a detoxifying effect is not a new concept. TRUE and OGLEVEE (4) found that glass, filter-paper, etc., segregated the more toxic components of certain aqueous solutions, thus lowering the concentration of these toxins in other regions of the solvent. They found also that dilute strong poisons are more affected than strong weak ones. This seems to be true for both electrolytes and non-electrolytes.

It would seem reasonable to anticipate a decided detoxifying effect or increased concentration at the interface of the adsorbent if the solute lowered the interfacial tension of the solution appreciably and the adsorbent carried a charge opposite to that of the toxin in aqueous solution.

Silica gel has been used commercially for adsorption of certain gases, but perhaps is not so generally used as activated charcoal for solutions. In this study of the action of substances modifying the toxicity of phenol, silica gel was chosen, not for its particular efficiency, but for the reason that it resembles to a degree one or more components of the more heterogeneous materials of which the soil is composed and to which plants have adapted themselves as a medium.

It is not known to what extent phenol occurs in soils, but it is reasonable to suppose that it is present to an extremely slight extent, if at all. We do know that conjugated forms of it in minute quantities are at times thrown off as excretory products of animals and as such may find their way to the soil.

Plants have adapted themselves to substances found most frequently in soil waters, and in a general way we may state that those elements which have a high solution tension and are also most abundant in the earth's crust are the least toxic. For example, we may contrast the slight toxic effect of the relatively abundant elements, sodium, potassium, and calcium with the more toxic effects of the rather rare elements, caesium, lithium and barium.

The same seems true of non-electrolytes (although more exceptions can be noted). Phenol does not occur in nature to any perceptible degree, but it is quite soluble. It exerts a marked degree of toxicity. Acetic and formic acid molecules are less toxic than cinnamic and hippuric molecules,

(TRUE, 5). Methyl and ethyl alcohol are less toxic than normal amyl, or normal hexyl alcohol, (EISENMINGER, 2).

The objects of the present study were: (1) to determine the relative toxicity of phenol at various concentrations in water solution; (2) to determine to what degree toxicity would be affected by the utilization of silica gel as an adsorbent; (3) to determine the effect on growth when mixtures of calcium nitrate and phenol in aqueous solution are used as the medium; (4) to determine the degree of adsorption of phenol in aqueous solution by silica gel.

Methods

As a criterion of toxicity the growth of roots of germinating soy-bean seedlings was used. The salts and organic matter of the seeds in the very early stages of growth are sufficient to prevent starvation.

The methods employed for determining growth rates were essentially the same as those used by TRELEASE and TRELEASE (3) and by EISENMINGER (1). The seeds were germinated on moist filter-paper in glass culture dishes in a dark room.

For each culture a Pyrex beaker of 300-cc. capacity (usual, or tall form without spout) was used. Over the top of this beaker was stretched a piece of paraffined mosquito netting which was secured below the rim by a ligature of paraffined linen thread. This beaker was then placed in a second Pyrex beaker of 600-cc. capacity and the culture solution was poured in until the liquid levels inside and outside the smaller beaker were even at its top.

When the primary roots of the seedlings had an average length of about 10 mm., seedlings were placed on the mosquito netting so that the roots dipped into the culture solution. Duplicate cultures, each of twenty-five plants, were used for each experimental solution.

The cultures were kept in a dark room, and the seedlings were allowed to grow until the primary roots of the control culture had acquired a length of about 98 mm., or until these roots had elongated about 88 mm. (98-10).

The length of the primary root of each individual plant was then recorded, and the average length of the roots of each culture was computed. From this length was deducted the average length of the roots when the seedlings were taken from the germinating dish. This difference constituted the average elongation of the culture. The growth data are relative values. Each was obtained by dividing the average elongation of a given culture by the average elongation of the control culture and multiplying by one hundred.

The average time required for the roots of the control solutions to acquire a length of 88 mm. was 90 hours. Four control cultures of 25 seed-

lings each were used. Also two distilled water cultures of 25 plants each were included in each series. The composition of the control solutions was as follows: 0.02 M KH_2PO_4 ; 0.02 M $\text{Ca}(\text{NO}_3)_2$ and 0.02 M MgSO_4 . The average temperature was 21° C.

The total concentration of phenol and of calcium nitrate was 0.006 M for one series of cultures and 0.06 M for the other. The beakers contained the following percentages of the total molecular concentration: 0, 2, 5, 15, 30, 50, 70, 85, and 100. In the solutions containing mixtures of phenol and calcium nitrate the following percentage proportions of 0.06 M of each component were used: 0 + 100, 2 + 98, 5 + 95, 30 + 70, 50 + 50, 70 + 30, 85 + 15, and 100 + 0.

When silica gel was used, 3 grams were placed in each beaker (300-cc. capacity) containing the various proportions of phenol. The contents of the beakers containing the phenol and gel were agitated at intervals during the period of growth.

The silica gel was of 200 mesh size. It had previously been washed until the filtrate gave no test for electrolytes.

In order to determine the apparent degree of adsorption, 300 cc. of the various concentrations of phenol—2, 5, 15, 30, 50, 70, 85, 95, and 100 per cent. of 0.06 M phenol in aqueous solution—were placed in a flask. In each of the flasks were placed 3 grams of silica gel for a period of time equal to the time the plants in the other series were growing. As before, the materials were agitated at intervals. The final concentration of the supernatant liquid was determined by adding an excess of standard bromine solution (Koppeschaar solution) to a portion of it, and titrating the excess of bromine with sodium thiosulphate.

Results

TOXICITY OF PHENOL

In the experimental data (table I) it will be noted that as the concentration of phenol becomes higher the toxicity increases—the relative growth becomes less. This is indicated by the percentage proportions of 0.006 M and of 0.06 M (figure 1A and 1B). When the concentration is higher than 0.03 M (50 per cent. of 0.06 M) scarcely any growth occurs.

GROWTH IN SOLUTIONS OF PHENOL TO WHICH SILICA GEL WAS ADDED

An examination of table I shows that solutions of phenol to which silica gel was added afforded better growth in nearly all concentrations than did the same concentrations of phenol without silica gel. This is likely due to the removal of some toxic principle. Since phenol is not highly ionized, the toxicity is not due to any great extent to the hydrogen ion.

TABLE I
GROWTH OF SOY-BEAN ROOTS IN TOXIC SOLUTIONS AND $\text{Ca}(\text{NO}_3)_2$

PROPORTIONS OF 0.006 M	AVERAGE RELATIVE GROWTH OF 50 SEEDLINGS EACH			PROPORTIONS OF 0.06 M	AVERAGE RELATIVE GROWTH OF 50 SEEDLINGS EACH			MOLECULAR PROPOR- TIONS OF 0.006 M MIXTURES OF Ca (NO ₃) ₂ AND C ₆ H ₅ OH		AVERAGE RELA- TIVE GROWTH OF 50 SEEDLINGS EACH
	C ₆ H ₅ OH	C ₆ H ₅ OH WITH SILICA GEL	Ca (NO ₃) ₂		C ₆ H ₅ OH WITH SILICA GEL	Ca (NO ₃) ₂	C ₆ H ₅ OH			
per cent.	mm.	mm.	mm.	per cent.	mm.	mm.	mm.	per cent.	per cent.	mm.
2	71.3	85.9	56.5	2	50.2	60.0	82.9	0	100	2.5
5	70.1	88.1	88.6	5	17.8	28.4	94.4	2	98	2.6
15	56.5	71.7	87.5	15	8.2	6.0	93.9	5	95	2.2
30	51.1	56.2	95.0	30	3.6	4.0	76.5	15	85	3.0
50	17.8	27.4	94.4	50	2.0	3.0	60.0	30	70	5.3
70	7.3	7.5	96.1	70	0	0	54.5	50	50	17.3
85	3.8	6.0	98.5	85	0	0	51.5	70	30	60.8
100	2.5	3.3	95.6	100	0	0	39.4	85	15	78.0
								100	0	95.6

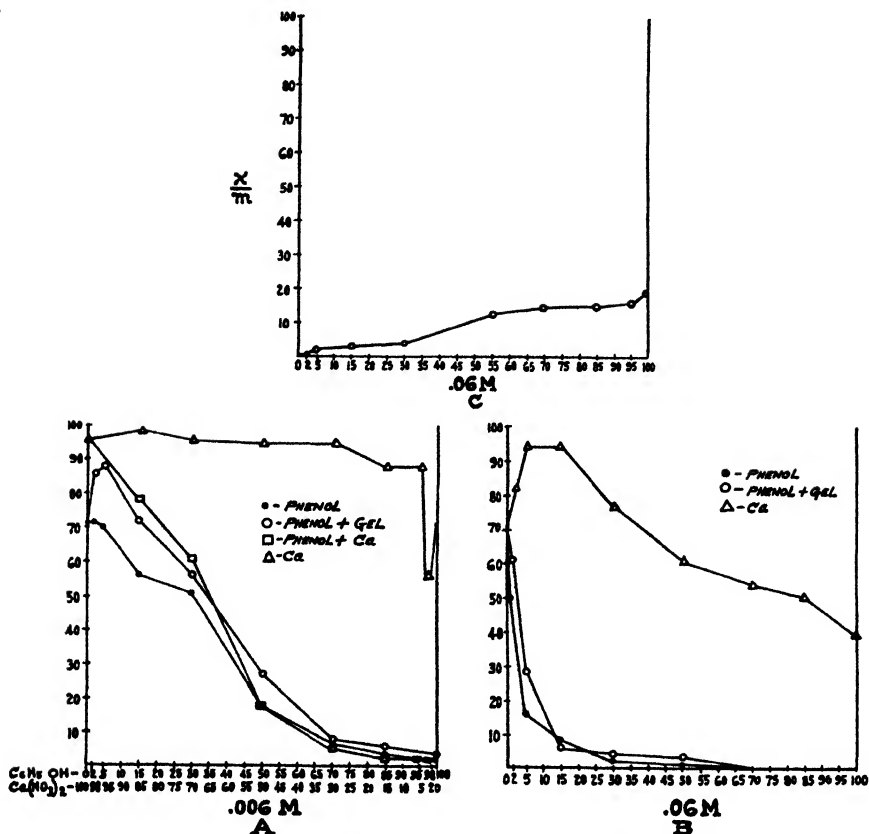


FIG. 1. A. Root elongation of seedlings in solutions of (1) phenol, (2) solutions to which three grams silica gel were added to each phenol solution, (3) solutions containing mixtures of phenol and calcium nitrate, (4) solutions containing calcium nitrate. Ordinates represent percentages of root elongation for standard solution; abscissas represent molecular proportions of 0.006 M. B. Root elongation of seedlings in solutions of (1) phenol, (2) solutions to which three grams of silica gel were added to each phenol solution, (3) solutions of calcium nitrate. Ordinates represent percentages of root elongation for standard solution; abscissas represent molecular proportions of 0.06 M. C. Adsorption of phenol by silica gel. Ordinates represent milligrams of phenol per gram adsorbent; abscissas represent percentage proportions of 0.06 M phenol.

At lower concentrations as in the percentage proportions of 0.006 M the culture solutions containing the silica gel afford an average growth approximately 8 per cent. better than those containing no gel. In the case of percentage proportions of 0.06 M this increased average is slightly more than 4 per cent. As the concentration of the phenol increases, the effectiveness of the gel to detoxify rapidly diminishes. At a concentration of 0.042 M growth is inhibited even though the silica gel is present. In

other words, at low concentrations of a toxic substance the media are relatively more sensitive to modifying agencies of this kind than at high concentrations.

TOXICITY OF CALCIUM

In the consideration of phenol we could regard the toxicity as the action of the molecule or a part of the molecule. In the case of calcium nitrate we are dealing for the most part with the toxicity of the calcium ion. Soybean seedlings are not decidedly sensitive to this ion; in fact, concentrations lower than 0.03 M seem to be less toxic to the seedlings than is distilled water. The roots of the seedlings grown in distilled water averaged only 71 per cent. of the length of those grown in control solutions. Low concentrations of calcium nitrate may serve to regulate the osmotic pressure which is undoubtedly a factor in distilled water toxicity. It is obvious, however, (figure 1B) that calcium ions are toxic at higher concentrations. When the concentration is equal to 0.042 M (70 per cent. of 0.06 M) there is a decided falling off in growth.

A solution of $\text{Ca}(\text{NO}_3)_2$ of concentration 0.018 M (30 per cent. of 0.06 M) is about as toxic as one of 0.00012 (2 per cent. of 0.006) solution of phenol.

TOXICITY OF MIXTURES OF PHENOL AND CALCIUM NITRATE

Mixtures of phenol and calcium nitrate throughout all proportions (figure 1A) are more toxic than the corresponding single solutions of calcium nitrate.

When the percentage proportion of phenol exceeds the percentage proportion of calcium nitrate of the 0.006 M mixture, the solution is slightly more toxic than either of the components used singly.

When the percentage molecular proportion of calcium salt exceeds the percentage molecular proportion of phenol, the mixture is less toxic than the corresponding single solution of phenol. The average growth in all the percentage proportions of calcium nitrate is approximately 54 per cent. better than in the corresponding solutions of the mixture.

It seems evident that when phenol has exerted its maximum toxicity no proportions of calcium salt can undo the effects—it has become a type of irreversible reaction. When the normal function of the cells is impaired but not totally disorganized, calcium may serve to remedy the harmful effects to a perceptible degree.

ADSORPTION

In an attempt to determine quantitatively the degree of adsorption, considerable difficulty was encountered at low concentrations. For the series, 2, 5, 15, 30, 50, 70, 85, 95, and 100 per cent. of 0.06 M phenol solution, values were obtained that seem fairly consistent (figure 1C). For the

same percentage proportions of 0.006 M no consistent increments were obtained by the analytical procedure.

For 300-cc. portions and 3 grams of silica gel with the above percentage proportions of 0.06 M the following values were derived:

M	GRAMS OF PHENOL PER 300 CC. SOLUTION	GRAMS OF PHENOL AD- SORBED BY 3 GRAMS OF SILICA GEL
0.0012	0.0338	0.00119
0.0030	0.0846	0.00660
0.0090	0.2540	0.00864
0.0180	0.5080	0.01087
0.0300	0.8467	0.04030
0.0420	1.1854	0.04171
0.0510	1.4394	0.04162
0.0570	1.6087	0.05024
0.0600	1.6934	0.05890

The average adsorption was approximately 3 per cent. of the total amount of phenol present. Silica gel adsorbed approximately 0.03 to 2 per cent. of its weight of phenol.

In view of the fact that adsorption does occur it seems more plausible to attribute the lessened toxic effects to lowered concentration of the media rather than to any oligodynamic phenomena due to retention by the silica gel of substances that could not previously be washed out.

Summary

The toxicity of phenol to plants increases with increased concentration.

Silica gel when placed in aqueous solutions of phenol lowers the degree of toxicity. When the concentration of phenol is increased the detoxifying effect is decreased.

At a total concentration of 0.006 M mixtures of phenol and calcium nitrate exert a toxic effect greater than that of calcium nitrate used singly. When the molecular proportion of calcium nitrate exceeds that of phenol, the toxic effects are less than those of the corresponding single solutions of phenol.

A portion of the phenol was adsorbed by the silica gel.

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STATISTICAL STUDY OF THE TOTAL NITROGEN IN BARTLETT PEAR SHOOTS¹

A. S. MULAY

(WITH FIVE FIGURES)

When working with biological material, especially trees, it is very difficult to adequately control all the external conditions. Internal conditions, either in plants or animals, are largely beyond control and beyond even such observations as may enable us to select a number of similar individuals. Thus the material to be studied is essentially heterogenous, which makes a statistical study of the material necessary for proper interpretation of the experimental results. The pertinent questions, answers for which are sought in the present study, are: (1) What is the magnitude of the natural variations? (2) How large a population should be taken to get results which will be representative of the group?

Material and methods

The material for this study was collected in the years 1927 and 1928. In 1927, fifty shoots were collected on the 4th of December from 50 uniform five-year-old trees at the University Farm, Davis. In the following year, shoots were collected on the 14th of November from thirty-year-old trees in a commercial orchard.² One hundred and fifty shoots were collected at random from a single tree and ninety from as many different trees. Some shoots were also chosen for the color of their bark; forty green and twenty-five brown shoots were collected from a single tree.

All the samples were separated into bark and wood, dried at 50° C., and ground to a fine powder. Another series of 272 shoots collected from a single tree was analyzed for total nitrogen without separating the bark from the wood.

The total nitrogen was determined by the simple Gunning method as described in the official methods of the A. O. A. C. (1).

Results

The results are presented as theoretical frequency curves (3) in figures 1 to 5, where observed frequencies are marked by circles. Figures 1 and 2 show frequency distribution of the bark and the wood total nitrogen in

¹ I am much indebted to Dr. J. P. BENNETT for his helpful suggestions throughout this work.

² This orchard is two miles south of Martinez, California, and has a valley climate. Thanks are due Mr. F. SWETT, owner, for his kindness in furnishing this material.

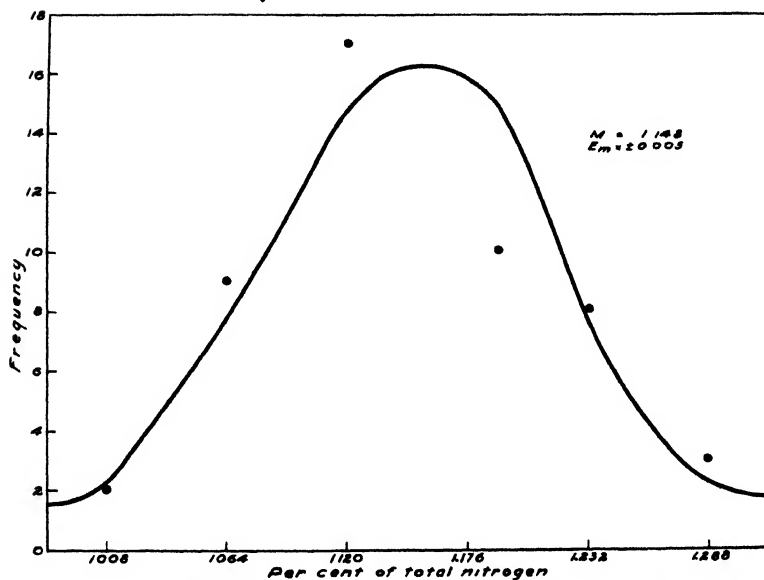


FIG. 1. Frequency distribution of total nitrogen in Bartlett pear 1927 bark from many trees.

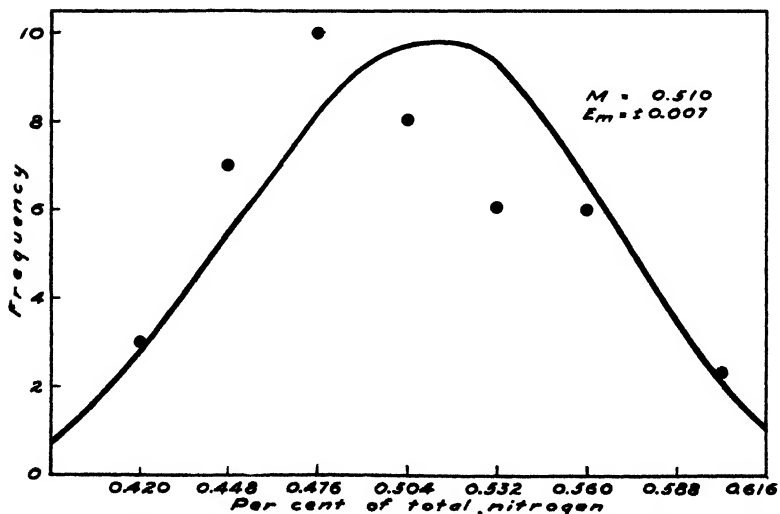


FIG. 2. Frequency distribution of total nitrogen in Bartlett pear 1927 wood from many trees.

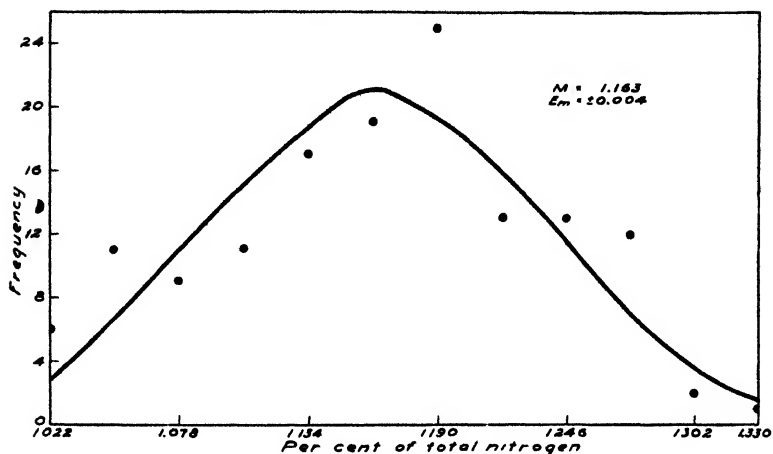


FIG. 3. Frequency distribution of total nitrogen in Bartlett pear 1928 bark from a single tree.

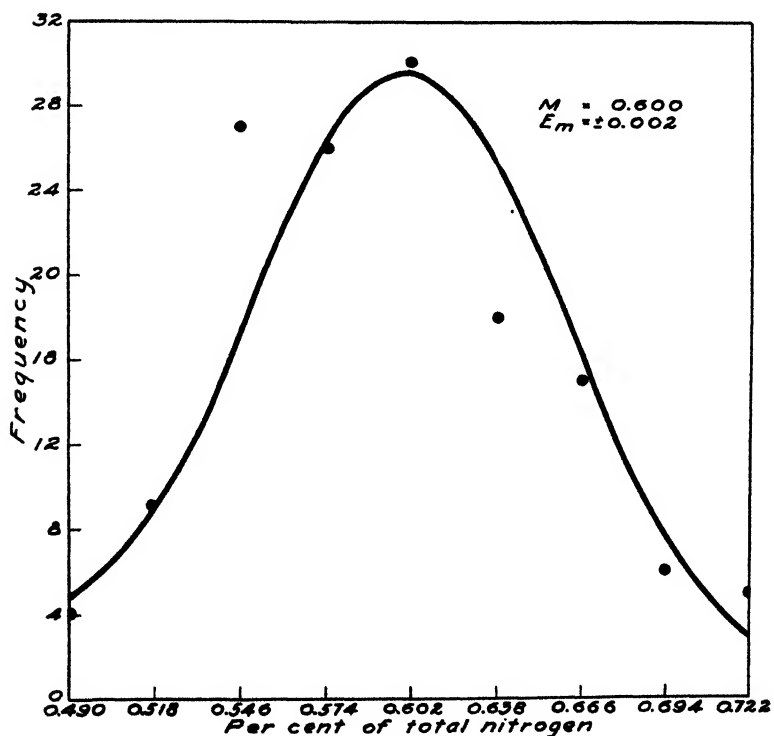


FIG. 4. Frequency distribution of total nitrogen in Bartlett pear 1928 bark from a single tree.

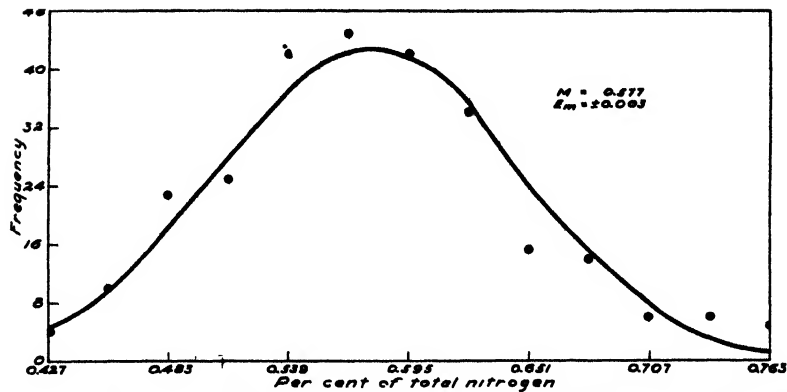


FIG. 5. Frequency distribution of total nitrogen in Bartlett pear 1928 twigs.

shoots from 50 different trees of the 1927 collection. Figures 3 and 4 give the nitrogen distribution in the bark and the wood from shoots collected at

TABLE I

NUMBER OF VARIANTS, MEAN, QUARTILE, AND NUMBER REQUIRED FOR DESIRED ASSURANCE, FOR THE DIFFERENT SERIES IN THE VARIABILITY STUDY

		SERIES	NUMBER OF VARIANTS	MEAN	QUARTILE	NUMBER REQUIRED FOR DESIRED ASSURANCE
From different trees	Davis 1927	Bark	49	1.148 ± 0.007	0.0456	10
		Wood	48	0.510 ± 0.005	0.0375	35
		Bark	87	1.225 ± 0.008	0.0440	8
		Wood	87	0.651 ± 0.006	0.0321	15
	Commercial orchard, 1928	Bark	149	1.163 ± 0.004	0.0498	12
		Green	40	1.077 ± 0.005	0.0304	5
		Brown	25	1.244 ± 0.004	0.0220	2
		Wood	149	0.600 ± 0.002	0.0349	22
		Green	40	0.585 ± 0.004	0.0244	11
		Brown	25	0.652 ± 0.005	0.0239	8
		Twigs	272	0.577 ± 0.003	0.0475	44

random from a single tree in 1928. Figure 5 shows nitrogen distribution of unseparated shoots from a single tree. As the results for the wood and the bark of the shoots collected from many trees in 1928 are similar to those for the shoots of 1927 they are not presented here.

Table I gives the number of variants, the mean, the quartile or P. E. Single (Q), and the number of shoots (N) required to assure the odds of 142:1 that the true value lies within ± 5 per cent. of the observed value. The formulae used in this connection were:

$$Q = 0.6745 \sqrt{\frac{\sum fd^2}{n-1}}$$

$$N = \frac{(\text{Coefficient of odds} \times \text{P. E. Single})^2}{\text{Deviation}}$$

Discussion

The results show that there is considerable difference in the extremes of the total nitrogen content both in the bark and in the wood of the pear shoots collected at the same time either from one or from many trees. Figures 1 and 2 for shoots from Davis show that the highest total nitrogen value for the wood is about 150 per cent. of the lowest, and the highest for the bark about 134 per cent. of the lowest. The bark of the shoots collected from a single tree shows differences (figure 4) similar to those in the bark of shoots from many trees, while the highest value for the wood from the shoots collected from a single tree (figure 3) is only about 136 per cent. of the lowest. The differences in the extreme values is much reduced both in the bark and in the wood when the shoots are selected by color. In the unseparated shoots this difference is about 80 per cent. of the lowest.

From table I it is seen that the bark and the wood of the green shoots have a smaller mean nitrogen content than the bark and the wood of the brown shoots. This shows that the total nitrogen content of the shoots varies according to the physiological age of the shoots and gives some ground for thinking that the differences in the total nitrogen content of the shoots collected at the same time may in part be due to the differences in their physiological age, though they may not show any visible evidence of this age.

Comparison of the quartiles in table I shows that the bark and the wood samples of 1927 are very similar to those of 1928 and in the 1928 samples those from many trees differ very little from those from one tree. The shoots collected from many trees are not more variable than those from one tree. The bark in general seems to be more variable than the wood, the quartile for bark being about 0.046, and for wood about 0.035. The shoots selected by color are less variable than those collected at random.

The quartiles for the green and the brown bark are 0.030 and 0.022 and those for wood from the green and the brown shoots are 0.024 and 0.024.

The last column in table I gives the number of shoots required to assure that the total nitrogen values observed are within ± 5 per cent. of the true value with the odds of 142:1. The number of the shoots required for this assurance is 8 to 12 for the bark, while for the wood it is 22 to 35. About 35 shoots from Davis pear trees are required to get a representative wood sample. As these values are not very precise, it is desirable, that whenever possible 40 or more shoots should be taken for a sample to assure a good representation.

Summary

Individual shoots either from the same or from different trees vary considerably in their total nitrogen content. The highest value for bark is 35 per cent. and for wood 50 per cent. higher than the respective lowest values. The shoots collected in 1927 are similar in their variability to those collected in 1928. The shoots collected from different trees do not vary any more than those collected from a single tree.

The bark and the wood from the green shoots are lower in their total nitrogen content than that from the brown shoots. This shows that the differences in the physiological conditions of the shoots may in part explain the variations in their total nitrogen content.

A sample of about 35 to 40 shoots is required to assure the odds of 142:1 that the true value lies within ± 5 per cent. of the observed value.

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COPPER AS AN ESSENTIAL FOR PLANT GROWTH¹

A. L. SOMMER

(WITH THREE FIGURES)

The stimulating effect of copper on plant growth was noted early in the use of copper salts as fungicides. A few years ago FELIX (3) obtained improvement in the growth of certain plants on several peat soils by the application of copper sulphate, both to the soil and in solution to the leaves. ALLISON, BRYAN, and HUNTER (1) were able, by the use of copper sulphate, to produce crops on certain otherwise unproductive peats of the Florida Everglades. Other treatments, notably caustic lime, manganese sulphate, and manure also gave improvement, but were not so beneficial as copper sulphate. BRYAN (2) also obtained greening in chlorotic leaves of plants grown in this soil by treating them with solutions of copper sulphate or manganese sulphate. These investigations do not furnish final proof, however, that copper is essential to plant growth. The work reported in this paper provides additional evidence on this point.

Sunflowers, tomatoes, and flax were used in these investigations. One-liter pyrex beakers with paraffine-coated, plaster of Paris covers were used as containers for the solutions in which the plants were grown. All water used in making up the nutrient solutions was redistilled from pyrex. In the first experiment with sunflowers the salts used had been repurified for an earlier study (5) on the effects of the absence of boron on plant growth. These salts had been recrystallized from water from a copper still; this still had the usual block tin condenser. In later experiments the water used for the purification of the salts was redistilled from pyrex. The methods used for the repurification of the salts² are described in a previous paper and will not be reiterated here.

The solutions to which copper was or was not added had the following composition:

¹ Presented before the Division of Biological Chemistry at the 78th meeting of the American Chemical Society, Minneapolis, Minn., Sept. 9-13, 1929.

² Analyses made recently in the laboratory of Professor FRED ALLISON (see *Journal of the American Chemical Society* 52: 3796-3806. 1930, for method) on samples of some of these salts showed that copper in a concentration of about 5×10^{-11} was present in the solutions because of copper added as an impurity of the KNO_3 , KH_2PO_4 , and MgSO_4 . Unfortunately samples of the other salts and the distilled water used were not available so that the total copper concentration of solutions to which copper was not intentionally added could not be determined. The amount added as impurities in the salts must, however, have been very small.

	per liter		per liter
KNO_3	0.80 gm.	$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.50 gm.
KH_2PO_4	0.15 "	CaSO_4 , saturated solution,	300 cc.

Iron was added in the form of FeSO_4 as the plants needed it. In the work with sunflowers, traces of the following elements were also added: manganese, aluminum, iodine, fluorine, sodium, chlorine, and boron. In addition to these, traces of tin, rubidium, lithium, barium, mercury, nickel, cobalt, arsenic, and lead were added to the cultures of tomatoes and flax. An excess of SiO_2 was added in all cases.

Dwarf sunflowers were used as experimental plants in the first investigation. The cultures were divided into two groups, one with and one without copper. Five cultures of two plants each were included in each group. Copper, as copper sulphate, was added to the solutions receiving copper at the rate of 0.125 mg. per liter. Two subsequent additions, one of 0.125 mg. and one of 0.06 mg. were made.



FIG. 1. Sunflowers grown with and without copper. Upper row with copper; lower row without copper.

The plants were transferred to the experimental solutions while in the cotyledon stage. By the end of the week, the plants in solutions containing copper began to show better growth than those without copper. Although very variable in size, those receiving copper appeared normal, and all were blooming at the time of harvest. Only one plant without copper produced a bud which was very small and appeared abnormal. The average dry weight per plant of those receiving copper was 4.2 gm.; that of the plants without copper was 0.31 gm. Plants grown with and without copper are shown in figure 1.

Sunflowers of a second series did not do so well. The reason for the poorer growth was not determined. Conditions which might have been causative, however, were: (1) the culture solutions became overheated when the temperature control of the conservatory was out of order, and (2) the salts used in the preparation of the nutrient solution were repurified from water redistilled from pyrex; consequently they may have been free from impurities which may have been beneficial to the plants of the preceding series. With the exception of one plant, those without copper were noticeably poorer within three weeks than those with copper. This plant appeared similar to the control plants for several weeks, and was as large as some of them when harvested. About the time it produced a bud, a little later than most of the control plants, it began to appear to be a very sick plant, and the bud did not develop. The average dry weight of plants without copper was 0.16 gm. Four plants were dead and the rest were in very poor condition. The average weight of plants with copper was 0.70 gm. The tops of these plants appeared normal, but the roots, like those of the plants without copper, were badly infected with fungi. Copper was added in this experiment at the rate of 0.06 mg. per liter. Four additions were made during the experiment.

Tomatoes (Dwarf Champion) were used as experimental plants in a subsequent investigation. This series was divided into two groups of six cultures each, one group with and one without copper. There were three plants to each culture. A single addition of 0.06 mg. copper per liter of solution was made to each of the six cultures with copper.

The plants were transferred to the experimental solutions in the cotyledon stage. All plants grew well for the first week. Soon after this, some of the plants without copper began to appear sickly; two were dead by the end of the second week; by the end of the third week a third plant had died. All plants with copper made good growth until the beginning of the seventh week, when one of the plants wilted; this plant was in very poor condition when the plants were harvested at the beginning of the ninth week. Most of the plants with copper had buds, and all except the one mentioned were in excellent condition. The average green weight

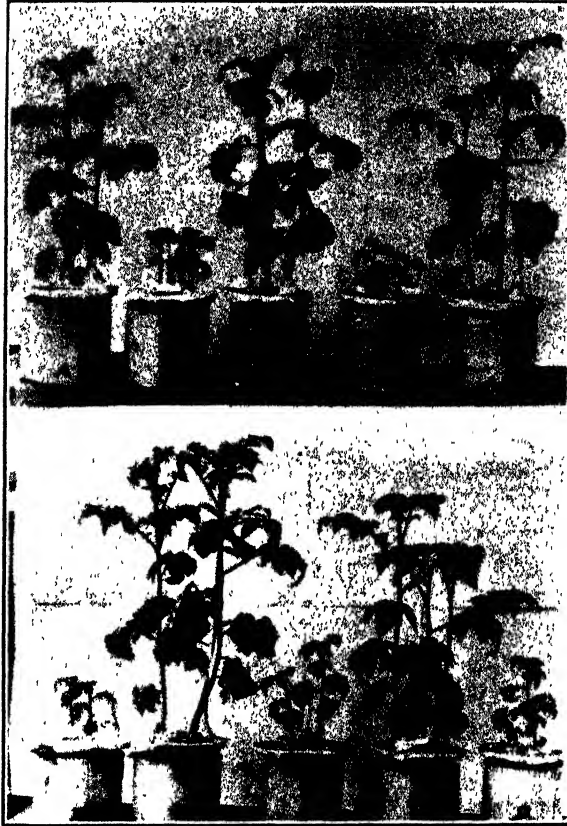


FIG. 2. Tomatoes grown with and without copper. Cultures (A) grown with copper; cultures marked (B) grown without. Only 0.06 mg. copper per culture was used.

of plants without copper was 2.9 gm.; that of plants with copper was 31.3 gm. The average dry weight of plants without copper was 0.3 gm., while that of plants with copper was 2.6 gm. The average did not include the three plants without copper which had died nor the one with copper which was badly wilted. The plants at time of harvest are shown in figure 2.

It is well known that copper is toxic to green plants even in relatively low concentrations. It was found by the writer in earlier work that there was considerable inhibition of root development for some plants at a concentration of 0.25 mg. per liter. It is, therefore, not surprising that a single addition of 0.06 mg. of copper would determine whether three plants would produce, as the largest culture did, 142.2 grams of green matter as compared with 12.8 grams, the weight of the largest culture without copper.

Moreover, it remains to be determined how small an amount of copper will be sufficient to produce an adult tomato plant.

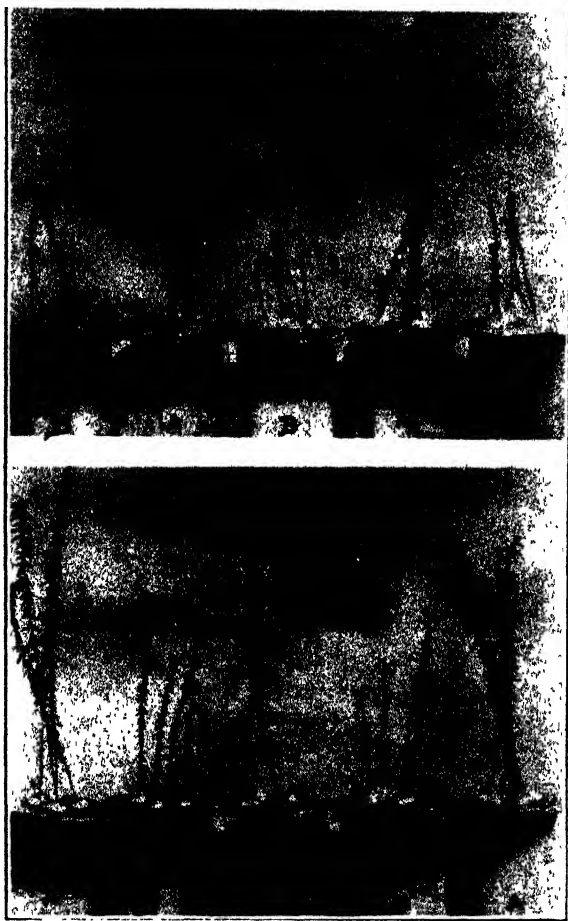


FIG. 3. Flax grown with and without copper. Cultures (A) grown with copper, cultures (B) without.

Flax was the third plant investigated. As in the previous experiments the plants were transferred in the cotyledon stage to the solutions to which copper had or had not been added. All plants grew well for the first week. By the middle of the second week the plants without copper were noticeably smaller than those with copper. About the end of the third week the roots of all plants appeared somewhat abnormal, and the plants with copper seemed to be growing more slowly than before. A second addition of 0.06

mg. of copper was made to each of the cultures with copper. Because this addition apparently was of no benefit, an addition of traces of all elements previously used in very small amounts was made to all cultures (cultures without copper as well as those with copper) a week later. The plants with copper began to show improvement within a few days, but those without it appeared to make no further growth. Six of the plants were dead at the time of harvest, fifty-two days after they were transferred to the experimental solutions. Most of the plants with copper had buds at this time but were not of normal size, as were plants grown at the same time in solutions of salts of ordinary purity. This indicates that flax has a larger requirement for one or more of the elements added in traces than does the tomato plant. The dry weight of eighteen plants without copper was 1.4 gm.; that of the same number of plants with copper was 4.5 gm. Plants grown with and without copper are shown in figure 3.

The investigations of FELIX, of ALLISON, BRYAN, and HUNTER and of BRYAN, even though showing very beneficial effects of applications of copper, did not furnish conclusive proof that this element is essential to plant growth. SMITH (4) has shown that there is a toxin in the black moor soils (Gliebe) of Holland which prevents normal plant growth, and which is rendered non-toxic by copper sulphate. It may be, therefore, that the beneficial effects obtained by the above mentioned investigators were due to a similar chemical action in the soils when the reagents were applied to the soils, or in the leaves when they were treated with solutions of these reagents. BRYAN showed greening of spots on chlorotic leaves where solutions of copper sulphate or manganese sulphate had been applied. A manganese deficiency is known to produce a certain type of chlorosis, but plants which were grown in solutions of purified salts to which no copper had been added were never chlorotic at any stage in their growth or decline. We have as yet no clew as to what the rôle of copper in plant metabolism may be, but the idea that it may act as an autooxidant is intriguing since it is well known that an extremely small trace of this element will act as a catalyst, greatly hastening the process of rancidity in fats. This catalytic property may also explain why both copper and manganese salts are so beneficial when applied to certain organic soils.

I wish to express my appreciation for the aid and encouragement which I received from the late Professor J. ARTHUR HARRIS in this work during my sojourn at the University of Minnesota.

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RELATION OF CATALASE ACTIVITY TO PHYSIOLOGICAL BREAKDOWN IN JONATHAN APPLES¹

J. R. NELLER

(WITH TWO FIGURES)

Considerable attention is being given to premature breakdown in apples for the reason that the disease constitutes a problem of both scientific and economic interest. This breakdown, which is physiological in nature, occurs in several varieties and has been described by several investigators (2, 3, 6, 10, 11, 12).

In the Pacific Northwest the Jonathan variety of apple is particularly susceptible to breakdown, which appears to be associated with amount of fruit per tree and the condition of the tree. However, the fundamental causes are still largely unknown.

If catalase activity may be considered as an index of the growth or nutritive condition of a plant as suggested by HEINICKE (4), and again by KNOTT (7), it may also be an index of the metabolic rate of activity in the flesh of an apple that is passing through a state of physiological breakdown.

Method of determination

An apparatus (figure 1) was designed that was somewhat different than that described by APPLEMAN (1). A water bath contained a wide mouthed reaction bottle of 60-cc. capacity; a three-hole rubber stopper fitted into this bottle, one hole being for the stirring mechanism, one for the ingoing hydrogen peroxide, and one for the outgoing oxygen. The glass stirrer was driven by a motor equipped with a variable speed reducer and was lubricated in its contact with the rubber stopper with a mixture of powdered graphite and glycerin. The hole in the stopper was cut larger than the glass stirring rod to enable a piece of rubber tubing to cover the rod where it passed through the stopper. It was found that a gas tight bearing was thus obtained that could easily be kept lubricated with the graphite glycerin mixture. As shown in figure 1 a hydrogen peroxide chamber, consisting of the bulb part of a 10-cc. pipette, was built into the system using two U-shaped glass T's. This enabled the operator to allow the hydrogen peroxide to mix with the catalase suspension after the entire system had been closed and the stirring started without changing the initial gas volume of the apparatus. During the course of the reaction

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FIG. 1. Apparatus used to determine catalase activity.

the leveling bulb was lowered occasionally so as to maintain an approximate pressure within the system. Readings were taken from the gas burette at one minute intervals with the use of a stop watch. All determinations were made at a temperature of 20°C . At first a record of air temperatures and barometric pressures was kept, but this was not continued as it was found that the greatest correction of the gas volumes thus obtained was considerably less than the variation between samples.

The work of various investigators has shown that the usual method of determining catalase activity by measuring the ability of the enzyme to decompose hydrogen peroxide results in an activity measurement of a relative value only, depending upon the conditions of measurement, such as method of preparing the sample, temperature of reaction, and H^+ ion concentration. Thus HEINICKE (5) found that it was necessary to neutralize tissues with low pH values before consistent catalase values could be obtained. OVERHOLSER (9) neutralized his pear tissue with calcium carbonate and found that what he terms the quantity of catalase obtained thereby was greater but in the same order as the catalase activity of a like set of unneutralized samples. The pH of his calcium carbonate treated samples ranged from 6.4 to 7.0.

In the present work it was decided to make all of the determinations in the presence of an excess of calcium carbonate. Samples were first prepared by using immediately a portion of the pulp obtained by passing the apples through a grater and then grinding in a mortar with calcium carbonate and quartz sand. It was found that the catalase activity was much lower than when portions of the same apples were ground in a mortar without passing through a grater. It was also found that there was much more catalase in the peel than in the flesh and very little in the expressed juice. Accordingly the following procedure was adopted. Apples were cut in halves (three to ten fruits per sample) with the line of cutting through the cheek and green side of the fruit. Thin triangular slices were then cut from the halves, care being taken to include the proper proportion of skin and core. A sample of twenty grams was macerated with five grams of precipitated calcium carbonate, adding enough water and quartz sand to facilitate grinding to a smooth paste. This was made up to 100 cc. and duplicate 20-cc. portions were pipetted into the reaction bottles which were placed in the water bath held at 20° C. The hydrogen peroxide was also held at a temperature of 20° C. in the bath. At first it was shaken with calcium carbonate before using, but it was found that this was unnecessary as no gas was given off thereby. After inserting the rubber stopper and stirrer the motor was started and the gas burette evacuated. The system was closed and kept under a vacuum of three or four inches of water for a half minute to make sure that there were no leaks. The 10 cc. of 3 per cent. hydrogen peroxide already introduced into the system were allowed to flow down into the catalase suspension and the stop watch was started. Gas volume readings were then taken every minute for four minutes or longer. Except for the July 2 determinations (table III) not more than 70 of the 100 cc. of available oxygen were needed to effect a cessation of gas liberation.

Experimental

The apples used in these experiments were grown in the Wenatchee Valley² and were put into cold storage at a temperature of about -1°C . as soon as harvested. Upon removal from cold storage they were immediately subjected to catalase determinations and analysis.

During the winter of 1928 preliminary experiments with fruit of the 1927 harvest established the standard procedure that was adopted as described above. In March of 1929 some catalase determinations were made on two lots of Jonathans of the 1928 harvest, one of which did and the other did not display the characteristic physiological breakdown. These show (table I) that catalase activity tended to be higher in the fruit that was breaking down.

TABLE I

RELATIVE CATALASE ACTIVITY OF NORMAL AND ABNORMAL LOTS OF JONATHAN APPLES OF 1928 HARVEST

SAMPLE	LOT 1*, SHOWING NO BREAKDOWN	LOT 2*, SHOWING BREAKDOWN	HALVES SEALED TO GLASS PLATES FOR 10 DAYS AT ROOM TEMPERATURE	
			FROM LOT 1	FROM LOT 2
	cc.	cc.	cc.	cc.
1	35.7	57.1		
2	35.1	39.7		23.0
3	46.5	68.5	67.8	

* Determined fresh from cold storage in March, 1929. Given in cc. of O_2 per 4 gm. tissue at 20°C .

Certain of the halves of the apples used for these determinations were sealed to glass plates with paraffin and held at room temperature for 10 days when they were analyzed for catalase activity. As shown in table I the halves of the sound apples of sample 3 had catalase values of 46.5 and 67.8 from cold storage and after 10 days at room storage, respectively, while corresponding values for the broken down apples of sample 2 were 39.7 and 23.0. Thus the catalase activity of the sound fruit, starting low, tended to go up and that of the broken down fruit, starting high, tended to go down when stored at an elevated temperature. This result supports the theory that physiological breakdown of Jonathan apples is associated with a high metabolic rate which later falls off below that of sound apples.

² The samples were kindly supplied by Prof. F. L. OVERLEY of this Station in connection with his production and storage studies.

An analysis (table II) of these apples as taken fresh from cold storage indicates that those in the process of breaking down tended to be somewhat higher in dry matter, sucrose, and nitrogen.

In a further study of the course of catalase activity some sound apples were subjected to catalase determinations when fresh from cold storage and after 26 and 43 days at room temperatures. Figure 2 shows that whereas

TABLE II

COMPOSITION OF JONATHAN APPLES FRESH FROM COLD STORAGE IN MARCH, 1929

SAMPLE AND CONDITION	TOTAL SUGAR	INVERT SUGAR	SUCROSE	ACID N 10 FOR 5 GM.	NITROGEN	DRY MATTER
	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>	<i>cc.</i>	<i>per cent.</i>	<i>per cent.</i>
Halves of breakdown lot showing least breakdown	10.69	8.76	1.93	2.6	0.048	15.81
Remaining halves of above, showing breakdown	11.02	8.45	2.57	2.2	0.046	15.98
Sound apples of above breakdown lot	10.58	8.90	1.68	3.2	0.049	15.34
Lot without visible breakdown	10.34	9.04	1.30	2.7	0.033	14.69

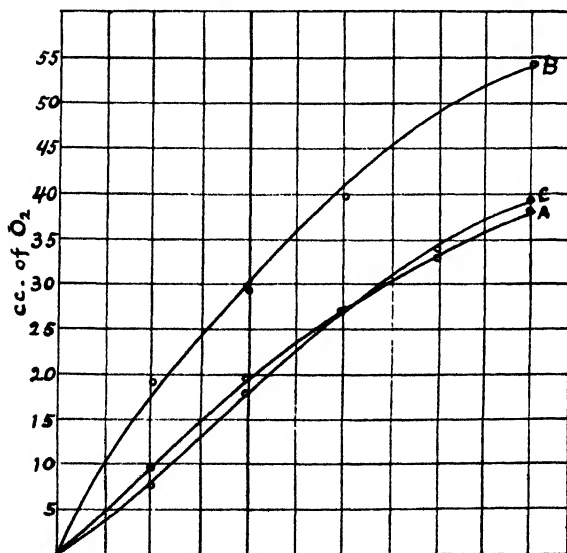


FIG. 2. Catalase activity of Jonathan apples; A as taken from cold storage; B after 26 days at room temperature; C after 43 days at room temperature.

the activity rate was considerably higher at the end of 26 days, it had dropped slightly below the initial rate at the end of 43 days. This rise and fall of catalase activity during the youth and senescence of the apples was probably more pronounced than the graph indicates, as gram equivalents were used at all dates and those after 43 days of room storage contained more dry matter than those fresh from cold storage. In this connection it is of interest to note that MAGNESS and BURROUGHS (8) found with an 18° C. storage that the catalase activity of apples that had been previously stored at 0° C. was higher than that of fruit previously stored at 1.7° C. OVERHOLSER (9) working with pears, has reported that prolonged storage at 20° C. caused the catalase activity to be much reduced over that of fruit stored at 0° C.

These findings caused an extension of the catalase determinations that were made on the next year's crop to include apples in advanced stages of breakdown. On five different dates (table III) samples of normal, broken

TABLE III

RELATIVE CATALASE ACTIVITY OF JONATHAN APPLES OF 1929 HARVEST FROM A LOT CONTAINING SOUND FRUITS AND FRUITS IN VARIOUS STAGES OF BREAKDOWN.*

DATE	SOUND APPLES	BREAKDOWN SHOWING	ADVANCED STAGES OF BREAKDOWN
	cc.	cc.	cc.
March 14 .	28.6	44.9	
April 8 .	32.1	40.0	27.2
April 30 .	33.3	46.7	12.5
May 15	40.6	57.8	11.1
July 2	68.9	91.7	13.5
Average	40.7	56.2	16.1

* Expressed in cc. of O₂ obtained in 4 min. from 4 gm. of tissue.

down, and badly broken down fruit were taken from cold storage for catalase determinations. As found in previous years catalase activity was considerably higher in the fruits that were showing breakdown. It was distinctly lower in fruits in advanced stages of breakdown. It may be observed also that the catalase activity of all samples tended to increase with increasing periods of storage with the exception of fruits in advanced stages of breakdown, which tended to decrease.

Summary and conclusions

A convenient type of apparatus for catalase determinations is described and a standard method of procedure is outlined.

As a result of a study extending over three seasons on the catalase activity of Jonathan apples as related to physiological breakdown, it was found that catalase activity tended to be higher in apples going through the breakdown process and to be decreased, below that of normal fruits, in fruits in advanced stages of breakdown. It was found also that the catalase activity of apples that did not develop breakdown tended to increase during the earlier and to decrease during the later periods of storage corresponding to the youth and senescence of the apples. These findings corroborate the theory that catalase activity measurements may be used as an index of the rate of metabolic activity. The data lead to the opinion that physiological breakdown in apples is associated with or caused by an accelerated metabolic rate.

The fundamental causes of physiological breakdown are still somewhat obscure. It is known, however, that the disease is most liable to occur when the crop on a tree is fairly small and the fruits correspondingly large (2).³ This is in line with the general tendency of fruit showing breakdown to be higher in per cent. of dry matter and sucrose.

If the breakdown is caused by an accelerated rate of metabolic activity a search for the cause might well include a study of physiological balance with regards to the food supply of the tree and its fruits. It is possible that more attention should be given to pruning and thinning operations so as to insure an optimum fruit load per tree. The complexity of interrelated factors that influence apple trees grown under intensive cultural conditions causes the study of physiological breakdown to be a difficult problem.

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IS ELECTRODIALYSIS USEFUL IN A STUDY OF APPLE TISSUE¹

J. R. NELLER

(WITH ONE FIGURE)

The separation of ions from colloids by the process of electrodialysis is a procedure that is being used with many types of materials. It has been especially favored since the introduction of the three compartment cell described by MATTSON (1).

MOORE, REEVES, and HIXON (2) appear to have been the first to employ this electrolytic method of dialyzing fruit tissue. They used the peels from Jonathan apples and found that samples from fruits affected with "Jonathan spot" reacted differently than those from normal fruits.

Experimental

During the course of a study of the cause of Jonathan breakdown (3) some samples were electrodialyzed in an endeavor to learn how the normal and abnormal apples might differ from each other. A three compartment cell was made much like the one described by MOORE, REEVES, and HIXON (2) except that instead of moulding a set of rubber sections, they were obtained by sawing a hard rubber battery box into three parts (fig. 1).

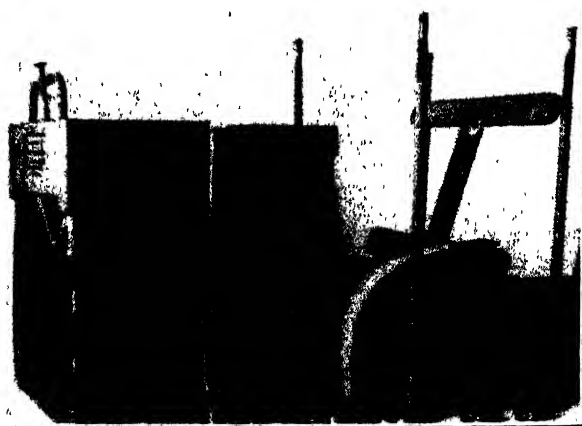


FIG. 1. Three-compartment electrodialysis cell, made from a hard-rubber battery box, with the rubber gaskets and clamping device.

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Four rubber gaskets were cut to fit the sawed sections of the box and one was put on each side of the parchment paper membranes. The cell became water tight when clamped together with four bolts.

Three hundred grams of apple tissue were put through a grater and suspended in 750 cc. of distilled water in the central compartment. A similar amount of water was placed in the cathode and anode compartments into which platinum coil electrodes were inserted. A potential of 110 volts was maintained for six hours, at the end of which time the cathode and anode solutions were drained from the cells and replaced with distilled water.

Temperatures taken in the central compartment at the end of each of the three six-hour runs per sample were found to average 62°, 47° and 40° C., respectively, and the corresponding current readings averaged 800, 630 and 275 milliamperes. At the beginning of each run the temperatures averaged 23° C. and there was a current flow of only a few milliamperes.

Table I shows that there was but little variation in the hydrogen ion concentration of the three extractions removed from the anode compart-

TABLE I

H-ION CONCENTRATIONS OF SOLUTIONS IN CATHODE AND ANODE CHAMBERS IN THE ELECTRODIALYSIS OF JONATHAN APPLE TISSUE

TYPE OF SAMPLE	ANODE COMPARTMENT			CATHODE COMPARTMENT		
	RUN 1	RUN 2	RUN 3	RUN 1	RUN 2	RUN 3
	<i>pH</i>	<i>pH</i>	<i>pH</i>	<i>pH</i>	<i>pH</i>	<i>pH</i>
Small apples, lot 1,* no breakdown present	3.10	2.88	3.35	Over 8	4.95 ^x	7.0
Large apples, lot 2, no breakdown showing	2.80	3.13	3.30	Over 8	6.86	7.0
Apples of above lot 2, with breakdown showing	2.83	2.88	3.34	Over 8	7.75	6.88

* Lot 1 and lot 2 apples averaged 115 and 180 grams respectively. Each run was of six hours' duration.

^x This solution stood over night in the cell with current turned off.

ment. In the cathode chamber the first extract was alkaline, while the second and third were practically neutral. pH values greater than 8.0 are not recorded for the reason that a quinhydrone electrode was used.

It may be observed (table I) that there was little difference in the hydrogen ion concentrations of extracts from normal and abnormal apples. These apples were distinctly different in that the normal lot 1, *viz.*, showing

no breakdown, were quite small, averaging only 115 grams per apple, having been unable to grow larger because of a restricted leaf area control,² while those of lot 2, were extra large and averaged 180 grams per apple.

TABLE II

ACID AND BASIC MATERIALS EXTRACTED BY THE ELECTRODIALYSIS OF JONATHAN APPLES
EXPRESSED AS CC. OF N/10 SOLUTIONS

TYPE OF SAMPLE	ACIDITY, ANODE COMPARTMENT				ALKALINITY, CATHODE COMPARTMENT			
	RUN 1	RUN 2	RUN 3	TOTAL	RUN 1	RUN 2	RUN 3	TOTAL
Small apples, lot 1, no breakdown present	cc. 55.0	cc. 61.0	cc. 14.0	cc. 130.0	cc. 110.0	cc. 6.0	cc. None	cc. 116.0
Large apples, lot 2, no breakdown showing	80.0	40.0	43.0	163.0	96.0	1.0	None	97.0
Large apples, lot 2, with breakdown showing	67.0	58.0	18.0	143.0	104.0	4.0	None	108.0

Neither were there any distinctive differences in the total amounts of acid and basic material extracted from these samples (table II). Somewhat more acid, however, was the extract from the normal apples of lot 2, but this was probably due to the fact that the tissues contained about 40 per cent. more total acid than those of the apples of lot 1.

An analysis of these extracts for NO_3 , Cl , SO_4 , K , and Ca ions showed (table III) that the only one of these ions that was extracted in any appreciable amount was potassium. Sodium and malic acid were not determined but must have been present in large amounts.

Since the ionic constituents of apple tissue consist largely of malic acid and its salts, a study was made of the dialysis of malic acid. Sugar and basic material were added to make the mixture more like that of apple tissue. This mixture consisted of 60 cc. of N/1 malic acid, 11 cc. of N/1 sodium hydroxide, and 30 gm. of grape sugar, the whole being diluted to 750 cc. These ingredients approximate those of the samples of apples discussed above.

After a six-hour dialysis of this mixture the values given in table IV were obtained. The acidity accumulation in the anode solution was higher than from the apple tissue samples due possibly to the presence of less adsorbing material in the synthetic sample. But since the pH value of the anode solution was considerably lower than that of a pure malic acid

² Dr. J. R. MAGNESS and Prof. F. L. OVERLEY kindly furnished these samples from their leaf area experiments.

TABLE III

IONIC CONCENTRATIONS FOUND IN EXTRACTS DIALYZED FROM APPLE TISSUE
EXPRESSED AS PERCENTAGE OF WEIGHT OF TISSUE USED

TYPE OF SAMPLE	NO ₃	Cl	SO ₄	K AS K ₂ O	Ca AS CaO	TOTAL ASH
		<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>		<i>per cent.</i>
Small apples, no break-down	Trace	0.0032	Trace	0.19	Trace	0.279
Large apples, no break-down showing	Trace	0.0015	0.0016	0.16	"	} 0.320
Large apples, broken down fruits of above lot	Trace	0.0026	0.0020	0.17	"	

solution of the same concentration which was found to have a pH of 2.65, it is believed that some active acidity was introduced as an impurity in the dextrose that was used.

TABLE IV

ELECTRODIALYSIS OF A MALIC ACID-DEXTROSE-SODIUM HYDROXIDE SOLUTION MADE UP TO SIMULATE
APPLE TISSUE FLUID

TOTAL ACIDITY IN ANODE CELL	TOTAL ALKALINITY IN CATHODE CELL	pH OF ANODE SOLUTION	CENTRAL CELL TEMPERATURE	CURRENT VALUES		pH OF RESIDUE IN CENTRAL CELL
<i>cc. N/10</i>	<i>cc. N/10</i>	<i>pH</i>	<i>° C.</i>	<i>milli-amperes</i>	<i>volts</i>	<i>pH</i>
151	44	2.03	55	800	100	2.70

At any rate, the results show that malic acid dialyzed fairly rapidly under the conditions imposed. The analyses given in table III, indicate that it accounted almost entirely for the acidity obtained in the apple tissue dialysis. Since there is so much malic acid in apple tissue, it seems that its progressive dialysis would mask any possible difference as to the amount, or manner in which it might be held, in normal and abnormal tissue. This would explain the similarity in acid extractions obtained from the normal and broken down fruits of the above experiment (table II). In this connection it is to be recalled that MOORE, REEVES, and HIXON obtained differences (2) in the rate of acid dialysis from normal apple peels and from those affected with Jonathan spot.

As found by these authors, the basic material that was obtained was removed during the early stages of dialysis (table II). While about the

same per cent. of malic acid was extracted from their samples, as in the present case. the percentage of basic material that they obtained was considerably higher. This suggests that much of the cation material of an apple is located in or near the skin. Table II, shows that about the same amount of basic material was obtained from normal apples as from those that were in stages of physiological breakdown.

These data tend to show that an electrodialysis separation is not useful in a study of the physiological breakdown of Jonathan apples. This does not mean that such fruits are not measurably, as well as visibly, different from those that are normal. For instance, it was found (3) that they differed in catalase activity and in composition. Since physiological breakdown results in the disintegration and death of the cells it is probable that factors, such as respiration and electrical resistance, are affected. Possibly also a different rate of electrodialysis than that described here would show a differentiation.

Summary and conclusions

A comparative electrodialytic study of normal and physiologically broken down Jonathan apples revealed no essential difference between the two types of fruits.

Extracts obtained from the anode compartment consisted almost entirely of malic acid. Only traces or very small amounts of nitrate, sulphate, and chloride ions were found to be present.

Extracts taken from the cathode compartment contained very little calcium and appreciable amounts of potassium, enough to account for over half of the total ash.

Practically all of the basic dialyzable material was obtained during the first six-hour period. The malic acid continued to dialyze out, but at a decreasing rate, throughout three six-hour periods.

The electrodialysis of a malic acid-sugar solution was similar to that of apple tissue itself.

The construction of a three compartment cell from a rubber battery box is described.

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BRIEF PAPERS

CHANGES IN OSMOTIC PRESSURE OF BANANAS DURING RIPENING

(WITH TWO FIGURES)

Various data are found in the literature indicating an increase in moisture content of the pulp of bananas during ripening. Of the physiological processes occurring, respiration causes the production of water. At the same time water is utilized in the hydrolysis of starch to sugar. As these two processes occur simultaneously, a part of the water produced by respiration may be used up in the hydrolysis of starch. GORE¹ has calculated the water formed by respiration and that utilized in the hydrolysis of starch, and has found that except when the banana becomes over-ripe, the water formed in respiration does not equal that used in hydrolysis. He also points out the probable changes in osmotic pressure with consequent transfer of water from peel to pulp.

The present communication deals with quantitative measurements of osmotic pressures of bananas and other parts of the bunch during ripening.

Experimental

In table I each value represents a composite sample of two fingers selected at random from the middle hands of three bunches of fruit (Gros Michel from Jamaica), which had been ripened at 68° F. and 90–95 per cent. relative humidity, in specially constructed ripening rooms. Peel and pulp were separated, ground in a mortar, and the cell sap expressed by centrifuging. The freezing point of the sap was determined according to regular cryoscopic technique. Corrections for undercooling were made from the formula:²

$$\Delta = \Delta^1 - 0.0125 U \Delta^1$$

where

Δ = corrected freezing point.

Δ^1 = observed freezing point.

U = degrees of undercooling below the
observed freezing point.

From the corrected freezing point, the osmotic pressure, in atmospheres, was calculated from the formula:²

$$P = 12.06 \Delta - 0.021 \Delta^2$$

¹ Gore, H. C. Changes in composition of peel and pulp of ripening bananas. Jour. Agr. Res. 3: 187–203. 1914.

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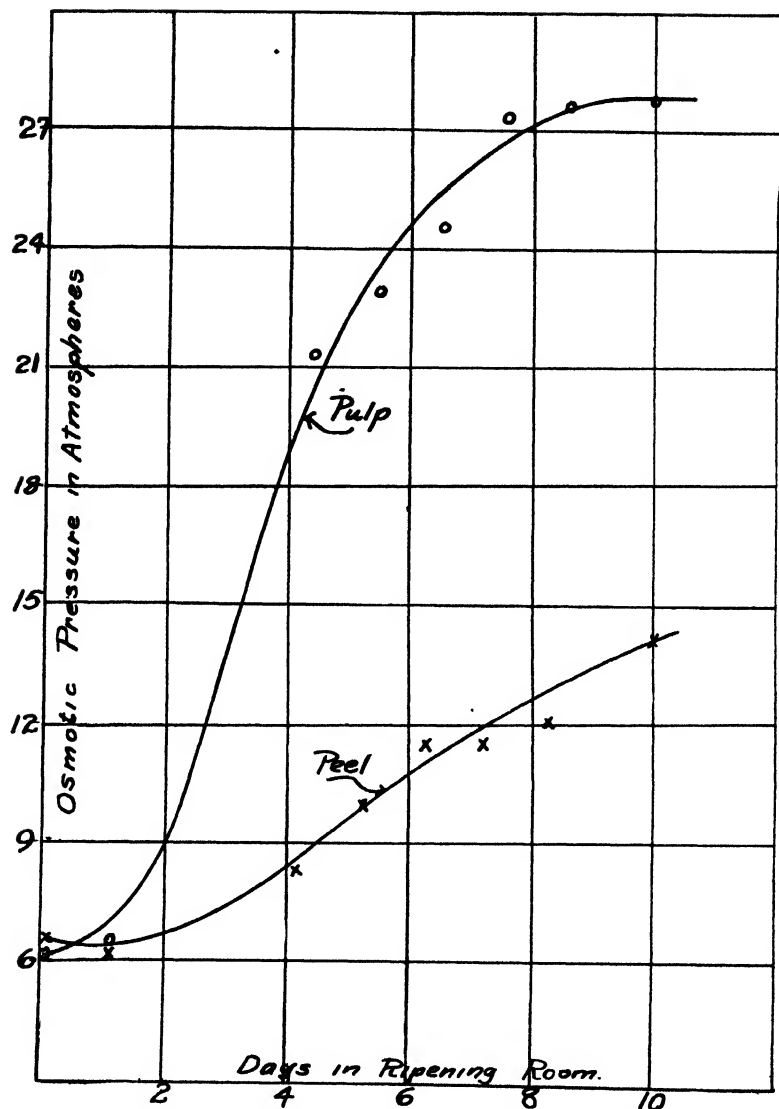


FIG. 1. Change of osmotic pressure of pulp and peel of bananas during ripening.

When two solutions of different osmotic pressures are separated by a semi-permeable membrane water moves from the region of less concentration of solute to that of the greater concentration of solute, with a tendency to produce equilibrium.

The results in table I show that there are marked changes in the osmotic pressure of the pulp and peel of the banana during ripening. At 0 days

(i.e. at the time of discharge from the boat at Boston) the osmotic pressures of pulp and peel are approximately equal and therefore at equilibrium, but as ripening progresses there is a marked increase of pressure in the pulp with a lesser increase in pressure of the peel. Differences in pressure between the peel and pulp naturally result in the migration of water from the former to the latter. These differences may be explained by the fact that while starch, the major constituent of the pulp of green bananas, does not affect the osmotic pressure to an appreciable extent, when this starch is hydrolyzed to sugar during the ripening process, the osmotic pressure then increases rapidly because of and in proportion to this production of sugar. The total sugars in the pulp amount to as much as 21 per cent. (unpublished data), while the sugar content of the peel is much lower with a maximum of about 4.4 per cent.

During the ripening period the fruit showed a loss in weight of 2.44 per cent. Furthermore the pulp increased from 68.5 per cent. of the total weight before ripening to 70.9 per cent. of the total weight after ripening, a gain of 2.4 per cent. GORE (*loc. cit.*) cites corresponding gains of from 2.24 to 3.90 per cent.

TABLE I

OSMOTIC PRESSURE OF PULP AND PEEL OF BANANAS DURING RIPENING

DAYS IN RIPEN- ING ROOM	COLOR OF PEEL	OSMOTIC PRESSURE			
		PULP*		PEEL*	
		I atm.	II atm.	I atm.	II atm.
0	Green	6.19	6.33	6.57	6.63
1	Starting to turn yellow	6.66	6.69	6.31	6.30
4	Very slight green	21.04	21.28	8.46	8.34
5	Green tip	23.70		10.38	
6	Full yellow	24.60		11.56	
7	Full yellow	27.40		11.56	
8	Speckled	27.87		12.39	
10	Speckled, fingers drop	27.88		14.68	

* Duplicate determinations were discontinued after the fourth day as the variation due to technique was shown to be negligible. (See figure 1 for graphical representation of above data.)

TABLE II

OSMOTIC PRESSURES OF DIFFERENT PARTS OF THE BANANA FRUIT BUNCH DURING
RIPENING

DAYS IN RIPENING ROOM	COLOR	OSMOTIC PRESSURE				
		PULP	PEEL	NECK	CROWN	STALK
		<i>atm.</i>	<i>atm.</i>	<i>atm.</i>	<i>atm.</i>	<i>atm.</i>
0	Green	7.15	6.19	7.17	7.82	6.81
3	Very slight green	16.91	9.29	7.41	6.55	6.10
6	Green tip	25.48	10.47	6.80	7.02	5.36
9	Slightly speckled	29.06	12.42	6.46	7.00	5.58

Commercial shrinkage studies have shown that cut hands suffered slightly greater shrinkage than hands ripened on the stalk. Our assumption was that a transfer of water from the stalk to the hands took place. In table II are presented our data from Gros Michel fruit (from Tela,

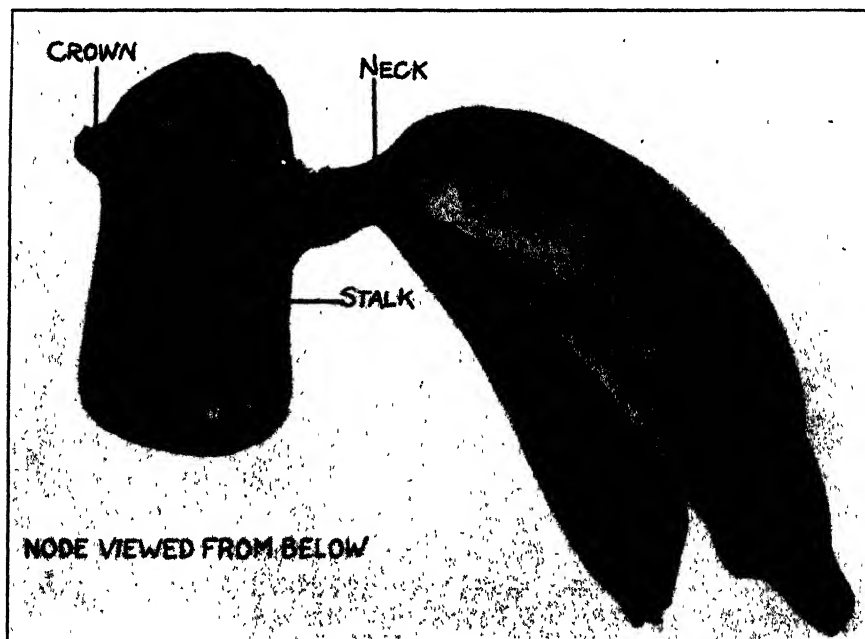


FIG. 2. Banana node, indicating parts tested in table II.

Honduras) ripened at 68° F., 90–95 per cent. relative humidity. Each value represents a composite sample from seven fingers, the necks of the corresponding hand (17 fingers), the corresponding crown, and a section of the stalk at the crown (fig. 2). The samples were crushed in a mortar, centrifuged, and filtered when necessary, to give a clear sap.

The results show that as ripening progresses there is, in general, a gradual decrease in order of osmotic pressures from the pulp to the stalk, indicating a transfer of water from other parts of the bunch of fruit to the pulp.

Summary and conclusions

Osmotic pressure determinations of different parts of the bunch during ripening of bananas indicate that changes of pressure are such as to bring about a transfer of water from peel to pulp, as well as from the stalk through the crown and neck to the peel and pulp.—FRANK C. STRATTON and HARRY VON LOESECKE, *Research Laboratories, United Fruit Company, Boston, Mass.*

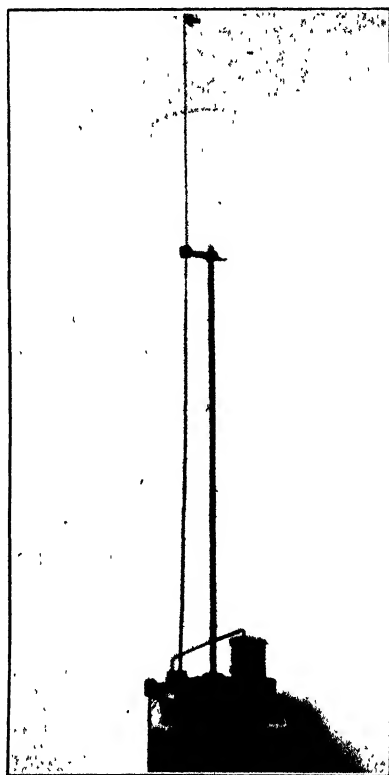
A QUANTITATIVE DEMONSTRATION OF OSMOTIC EQUILIBRIUM

Demonstrations of the diffusion of solutes and solvents through differentially permeable membranes are common in all branches of natural and biological science. Ordinarily the membrane used is so imperfect, however, that most of the value of the demonstration is lost and the students do not obtain a concept of osmotic pressure as a diffusion equilibrium dependent upon the relative activity of solute and solvent on the two sides of the membrane. The following modification of the PFEFFER technique for measuring osmotic pressure has been used in our laboratory for several years. It has the advantage of requiring no special equipment or technique to give quantitative osmotic pressures for sucrose solutions at concentrations between 0.01 and 0.10 molar, with an accuracy within two to four per cent. of the calculated values by the FINDLAY or other corrected equations.

The apparatus is set up as shown in the figure with a reservoir manometer and a copper ferrocyanide membrane deposited within the walls of a Livingston cylindrical atmometer cell. The primary difficulties are the maintenance of an unbroken copper membrane on the *inside* of the porous cup, and adequate wiring or clamping of the rubber stoppers to withstand the pressures developed.

The membrane is formed by drying a new, unshellacked atmometer cylinder at 100° C. An old cylinder may be used if the shellac is removed

and it is cleaned with ammonia and nitric acid and washed for several hours in running water. The dry tube is covered with 0.5 M copper sulphate solution and the air removed by repeated evacuation. The saturated tube is then quickly wiped inside and out with a clean cloth and filled within one-fourth inch of the top with 0.25 M potassium ferrocyanide solution which also contains the desired concentration of sucrose. The two solutes should be mixed and made up to volume together. The ferrocyanide solution should not come in contact with the outside of the cell, for a double membrane will be formed which may develop defects. A



rubber stopper with a piece of capillary glass tubing to connect the cell to the manometer is inserted and carefully wired into the cell. A metal washer slightly smaller than the stopper will prevent the wires being forced into the rubber. Special screw clamps may be used for holding the stopper but they are more likely to break the cell than is the wire. If a soft iron or copper wire of medium size is used it may be cut into short lengths and five to ten of these laid fanshaped over the top and bent down

past the shoulder of the cell on both sides. The ends of these wires are then tied in under the shoulder, drawn tight, bent up and tied a second time. The cell and tube should be filled with solution, although small air bubbles will do no harm.

A small quantity of mercury is placed in the bottom of the reservoir, the bottle filled with water and a two-holed stopper, containing the cell connection and manometer, wired in. The osmotic cell is then completely immersed in 0.5 M copper sulphate solution and, as soon as diffusion starts, mercury is added to the manometer tube until equilibrium is reached. A 4 to 6 mm. manometer tube should be used so that mercury can be added from the top. This avoids dilution of the test solution through inward diffusion of water.

Five-tenths molar copper sulphate is roughly isotonic with 0.25 M potassium ferrocyanide, but for accurate work a blank determination is made and subtracted from the readings for the test solutions. The presence of the salts insures a good membrane and the ferrocyanide also acts as a preservative for the sucrose.

The setting up of the apparatus requires some manipulative skill, but pressures of an atmosphere are demonstrated readily and pressures up to two and one-half atmospheres have been produced in our laboratory. Properly constructed membranes are so slowly permeable to sucrose as to hold 80 per cent. of the maximum pressure after three months.—W. E. LOOMIS, *Department of Botany, Iowa State College, Ames, Iowa.*

NOTES

Pasadena Meeting.—The American Association for the Advancement of Science holds a summer meeting at the California Institute of Technology, in Pasadena, June 15–20, 1931. The American Society of Plant Physiologists will participate in this meeting to a limited extent. A program is being arranged under the leadership of Dr. D. R. HOAGLAND, of the University of California. The attention of our western members is directed to this opportunity for mutual encouragement, and for the discussion of progress in research. These summer meetings may be somewhat more local in character than the winter meetings, but they will provide opportunities for less expensive attendance at the meetings for those who happen to reside within a short distance of the meeting places chosen. It is hoped that the western group of members will respond cordially to the efforts of the program committee for this summer gathering.

Council Representatives.—President H. R. KRAYBILL has appointed Dr. C. A. SHULL, of the University of Chicago, and Dr. GEORGE J. PEIRCE, of Stanford University, to represent the American Society of Plant Physiologists on the Council of the American Association for the Advancement of Science, during the coming year.

Memorial Committee.—At the Cleveland meeting of the Society it was decided to appoint a committee to arrange for special features of our annual meetings, with special reference to the commemoration of anniversaries of the great leaders in the field of plant physiology during past epochs. The Cleveland meeting was dedicated to JAN INGEN-HOUSZ, who was born December 8, 1730. It would have been very appropriate to have had a more elaborate program in memory of the 200th anniversary of his birth. It was felt that future events of this kind might be commemorated by special meetings devoted to the different phases of influence which these men have exerted upon the development of plant physiology in their own time and ours. It was also decided that the meeting of 1932 should be made commemorative of the life of JULIUS VON SACHS, the 100th anniversary of whose birth occurs in that year.

The Memorial Committee provided for has now been appointed by the President of the Society as follows: Dr. C. F. HOTTES, University of Illinois, chairman; Dr. C. O. APPLEMAN, University of Maryland; and Dr. FRANK M. ANDREWS, Indiana University.

Program Committee.—President KRAYBILL has also appointed the Program Committee for the New Orleans Meeting in December, 1931. The members chosen for this service are Dr. E. S. REYNOLDS, the Missouri

Botanic Garden and Washington University, chairman; Dr. D. B. ANDERSON, North Carolina State College; Dr. L. J. PRESSIN, Southern Forest Experiment Station; and Dr. C. B. LIPMAN, University of California. The Secretary, Dr. W. A. GARDNER, Alabama Polytechnic Institute, is also *ex officio* a member of the program committee.

Membership Committee.—The Membership Committee appointed to serve during the ensuing year will appreciate the cooperation of all members in their efforts to reach all of those who need the literature of plant physiology in their work. Dr. R. E. GIRTON, of Purdue University, is chairman of the committee, and serving with him are Dr. J. P. BENNETT, University of California, and Dr. LEE HUTCHINS, of the Bureau of Plant Industry, U. S. D. A., Washington, D. C. All applications for membership receive prompt attention from the Executive Committee.

Purdue University Section.—During the last year the Purdue Section of the American Society of Plant Physiologists has had its best year so far, a membership of 38, and an average attendance of 29. The meetings occur at 4:00 P. M. in Stanley Coulter Hall. The 1930-1931 program will be interesting to all of our members. It shows what an active group can do with the privileges of sectional organization. The entire program consisted of 13 meetings, with the following addresses:

- October 14, (Dinner meeting) The Fifth International Botanical Congress, by Dr. J. C. ARTHUR
- October 20, Effect of mineral nutrition on development of rusts and mildews, by K. D. DOAK
- November 3, The equipment and arrangement of the plant physiology laboratory for teaching and research, by R. E. GIRTON
- November 17, Some interesting phases of insect metabolism, by W. A. Hiestand
- December 1, Country life in Scotland, Prof. AITKENHEAD
- December 15, Laboratory versus field germination tests in soybeans, by G. H. CUTLER
- January 5, 1931, Reports on the Cleveland meetings of the A. A. A. S., a joint evening meeting with the Biological Society
- January 19, Light and some of its effects upon plants, by G. E. READ
- February 2, Factors affecting hardiness in peach buds, F. P. CULLINAN
- February 16, A physiological consideration of the ontogeny of leaf, root and stem, by E. J. KOHL
- March 2, Bacterial decomposition of cellulose at high temperatures, by P. A. TETRAULT
- March 16, Protein synthesis in plants, by H. R. KRAYBILL
- April 6, (Dinner meeting). Dr. E. N. TRANSEAU, Ohio State University

Such meetings must prove to be helpful and stimulating in the development of a high grade research atmosphere in an institution. The example is worthy of emulation by others. At the meeting on March 2, officers for 1931-1932 were elected. Chairman, Dr. LAURENZ GREENE; Secretary, Dr. E. J. KOHL.

Agricultural Books.—Baillière, Tindall and Cox, 7 and 8 Henrietta St., Covent Garden, London, W. C. 2, offer their services to any of our members who may have need of Agricultural Books, either old or new. They will send their catalog to any one who may be interested in acquiring works of this type, and will give courteous and prompt attention to all orders which may be sent to them.

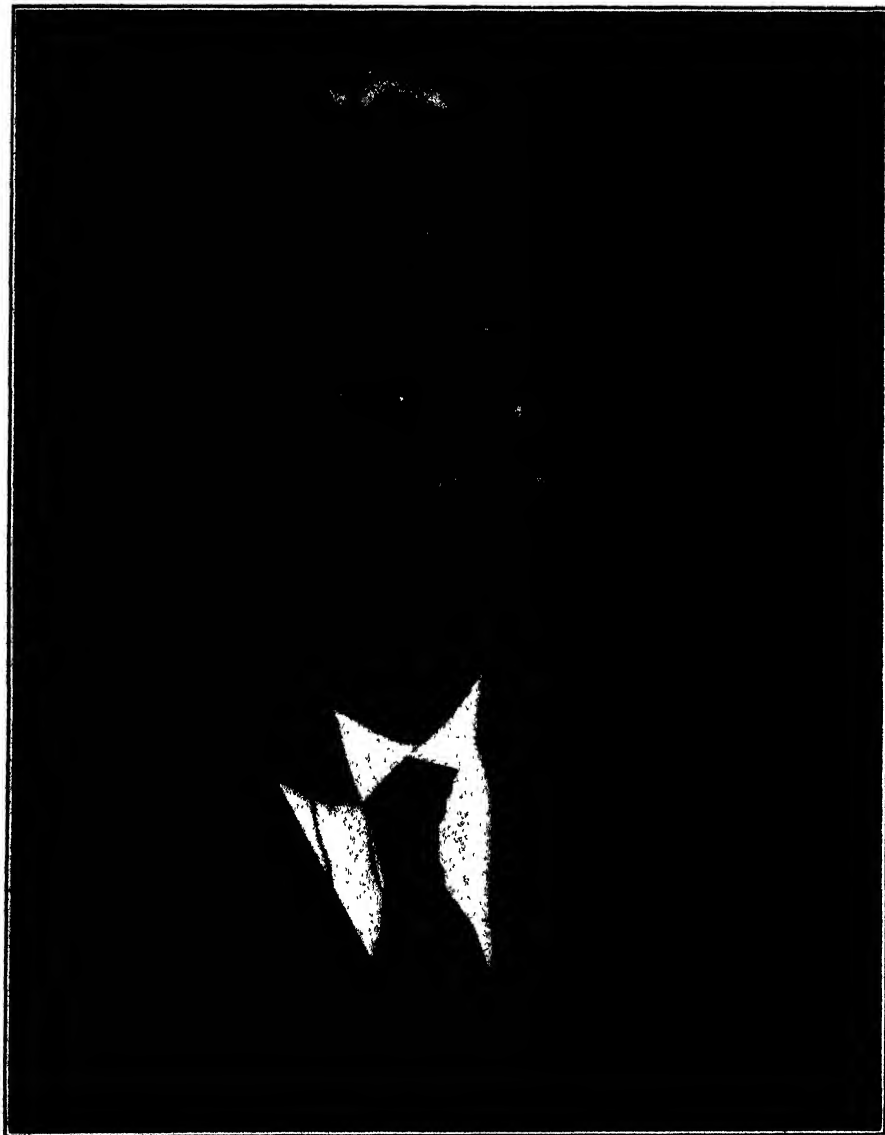
Portraits.—We are fortunate to be able to present in this number a portrait of Dr. F. F. BLACKMAN, of the Botany School, Cambridge, from a crayon of unusual merit by one of Dr. BLACKMAN's students, Miss DE BIDEN FOOTNER, in July, 1924. All portraits are obtainable from the editor at Chicago at 12 cents each, or in complete sets of 13 for \$1.45 in postage. The number printed is limited, as the demand has not been large. Laboratories should avail themselves of the opportunity to obtain these prints before any of them become exhausted.

Collected Works of Emile Godlewski.—The Academie Polonaise des Sciences et des Lettres has published Vol. I of the collected works of EMILE GODLEWSKI, père, the beginning of a three volume edition of his complete works. This publication was undertaken to celebrate GODLEWSKI's 80th birthday. The value of GODLEWSKI's work to the fields of agronomy, agriculture, and plant physiology makes this collection of his contributions unusually worth while. The first volume contains 599 pages, and covers the period from 1870-1890. The second volume will contain the papers appearing from 1891 to 1910; and the last volume, the recent work, since 1910, particularly his popularizations of agricultural science. The papers are printed in the original language, but where printed only in Polish, summaries in other languages, or translations, will be provided. The first volume has a fine portrait of GODLEWSKI, and a facsimile reproduction of a page from one of his manuscripts, a plant physiology which has not yet been published. There is also an autobiography in volume I, and the last volume will have a bibliographical index of his complete works. These three volumes will be a splendid monument to GODLEWSKI's industry and achievement in research. Inquiries for the work should be addressed to the Academie Polonaise des Sciences et des Lettres, 17 Rue Slawkowska, Cracovie, Poland.

Handbook of Plant Nutrition.—A voluminous work in two volumes in this field is announced by Julius Springer, Linksstrasse 23–24, Berlin W9. Its title is *Handbuch der Pflanzenernährung und Düngerlehre*, and volume II, *Düngemittel und Düngung* has come out ahead of volume I, *Pflanzenernährung*, which will appear later in the spring of 1931. The editor of the work is Dr. F. HONCAMP, Director of the Experiment Station at Rostock, who has had the assistance of 50 cooperators in producing the work. The material of volume II is presented in nine chapters, the first of which outlines the historical background, and the general influence of fertilizers on soils and plants. The next three chapters deal with the natural and artificial fertilizers, and their application. Chapter five gives an account of fertilizer practice with specific crop plants, some 25 different agricultural species being considered in detail. The later chapters discuss forestry fertilizer problems, fertilization of moor and heath soils, ponds, and the use of fertilizer materials in control of disease, animal pests, and weeds. With the index, it is a volume of 919 pages. The price of volume II in paper cover is 86 RM, and with cloth binding 89.8 RM. It seems to be a valuable addition to the literature of nutrition of agricultural plants.

Principles of Agrobiology.—This little book, by OSWIN W. WILCOX, claims to be the only work which “gives an orderly and adequate statement of the axiomatic fundamentals of crop growth.” It is also claimed that agriculture can now be, in the hands of the ablest practitioners, an exact art. The booklet contains less than a hundred pages. Part I presents a discussion of the primary laws of agrobiology, upon which agriculture as an exact art, is predicated. These are: Law of the constancy of type, law of definite growth powers, law of the universality of essential growth factors, law of the constancy of action of growth factors, law of the joint action of growth factors, law of definite optima, law of diminishing increments of yield, law of increasing increments of yield, the concentration law of growth factors, and the logistic law of crop growth. Part II considers the general law of growth factors and its derivatives; and part III, fertilizer statics. There is a brief mathematical appendix.

The author has copyrighted his ideas, which will militate against any general use of them. The book is published by the Palmer Publishing Corporation, New York. The price is \$4.00 per copy.



FRIEDRICH AUGUST FERDINAND CHRISTIAN WENT

1863

PROFESSOR OF BOTANY AND DIRECTOR OF THE
BOTANIC GARDEN

AND

INSTITUTE OF GENERAL BOTANY
UNIVERSITY OF UTRECHT

PLANT PHYSIOLOGY

JULY, 1931

ABSORPTION OF MINERAL ELEMENTS BY PLANTS IN RELATION TO SOIL PROBLEMS¹

D. R. HOAGLAND

The discussion which I wish to present falls in the general field of research which can be designated by the phrase "Soil Conditions and Plant Growth," to quote the title of a well known monograph by E. J. RUSSELL. An attempt will be made on this occasion to give some consideration to both soils and plants, with particular reference to researches of the past fifteen years. I am aware that some of our most important knowledge of soils has come from those who have dealt with the soil as a chemical and physical system without much reference to plants, and that much of what we know concerning plant physiology has come from investigators not greatly interested in soil conditions. Nevertheless, there does remain an indispensable place for investigations in which specifically planned efforts are made to relate soil conditions to plant growth, the ultimate purpose of all the researches from an agricultural point of view. In California the desirability of conducting such researches was stressed early by C. B. LIPMAN, and various circumstances, partly accidental, have made it possible to give long continued attention to this type of investigation. With your permission I shall refer frequently to experiments with which I have had some personal familiarity including, of course, the work of various colleagues and associates. I assume that you are not now desirous of a review of literature or an extensive citation of the names of the many investigators over the world who have contributed to our understanding of soil and plant relations.

If we are to review the subject of the absorption of mineral elements by higher plants, it is well first to inquire which chemical elements must be absorbed in order to insure the continued growth of such plants. The pioneer researches of MAZÉ very definitely suggested that the older list of

¹ STEPHEN HALES address, Cleveland, 1930.

essential elements was incomplete. Unfortunately these researches did not have much effect in stimulating other investigators until within a very recent period. The interest in this question is now aroused and in various laboratories in this country and in Europe very painstaking experiments have been in progress for some time. Eventually there may be many elements to add to the older list of ten. Several can be added now with much assurance, not necessarily for all plants, but for many types of agricultural plants at least. The importance, and in fact necessity, of considering a new and extended list of essential elements is convincingly illustrated by certain experiments on tomato plants, in connection with the utilization of boron. A few milligrams of this element make the difference between a healthy and a diseased plant. To prove this it may not be necessary even to purify the salts used in preparing the culture solutions. The amount of boron required, although very small, is still appreciable and the amount present as an impurity in a culture solution prepared in the routine way may be entirely inadequate for some plants. Of great interest, likewise, is the general distribution of copper in plants and the influence of this element in correcting certain diseases of fruit trees and other plants. While such observations cannot prove that copper is an essential element, they do offer an incentive toward additional research. We cannot go farther in our present discussion of such elements for almost nothing is known about their absorption from soil solutions or from artificial culture solutions. It would be difficult indeed to make some of the requisite experiments, but the task must be undertaken sooner or later, if the understanding of soil and plant relations is ever to become reasonably adequate.

The next phase of our discussion in logical order is concerned with the general nature of the processes by which plants absorb essential or non-essential chemical elements from culture media. A clear approach to this question is frequently impeded by the indiscriminate use of certain common terms such as permeability, osmosis, diffusion, antagonism, DONNAN equilibrium, etc. At the risk of repetition of previous discussion I intend to review very briefly certain of the conditions of plant growth which demand consideration in any survey of the process of absorption of mineral elements by plant cells. As you know, during recent years several investigators have given much attention to the behavior of certain types of algal cells which attain relatively enormous size, and which may be manipulated as separate units. With cells of this type it is possible to avoid many of the difficulties and ambiguities inherent in the examination of the microscopic cells and complicated tissues of higher plants. The investigations on the cells of the marine form, *Valonia*, yielded data of great significance with respect to the relations existing between a marine organism and its

solution environment. Our own special interest has been directed to experimentation on a fresh water alga, *Nitella clavata*. These plants live and grow in a medium which may be reasonably compared in a general way with many soil solutions. Also it is feasible to subject these cells to the influence of artificial culture solutions and to make experiments on the intake of solutes under controlled conditions. For present purposes such a cell may be regarded as essentially constituted of a cell wall, an exceedingly thin layer of protoplasm, and a large vacuole filled with sap primarily inorganic in nature. It is found that this sap has a total electrolyte concentration approximately twenty-five times greater than that of the water which bathes the cells, and furthermore, each of the principal ions (both anions and cations) is present in the sap in a concentration many-fold that of the culture medium. Evidently, during the growth of the cell there occurs an accumulation of such elements as potassium and chlorine against concentration gradients. For certain elements the same statement can be made for *Valonia*, and very recently a member of the *Characeae* living in brackish water has been studied in Finland, and similar conclusions reached.

Experiments on *Nitella* cells showed that the process of concentrating electrolytes in the cell sap could be conveniently studied by the use of bromide solutions, since bromine was not naturally present in the cell sap in determinable amount, and was non-toxic in the concentrations used. To make a long story short, it was found that bromine could be accumulated in the sap against concentration gradients, but only provided the right metabolic conditions were maintained. Important among these conditions was illumination. Any large accumulation of bromine was dependent on the influence of light of suitable intensity and duration, although under certain circumstances an after effect of illumination could be demonstrated. The temperature coefficient of the process was a high one, and little or no concentrating action was observed at temperatures approaching 5° C. The time factor was important and to obtain marked accumulation required a period of several days. Experiments of very short duration would be inadequate to reach an understanding of the processes involved.

Later F. C. STEWARD investigated the accumulation of bromine and potassium by potato tissues. In this case, of course, stored carbohydrates were available and photosynthesis was not directly involved. The potato tissues also were found to possess the potentiality for concentrating in the sap the elements mentioned above. However, it was discovered that the concentrating process could not proceed unless the tissues were in a healthy state and suitably aerated. When a mixture of oxygen and nitrogen with

a very low proportion of oxygen was employed the concentration of bromine in the expressed sap exceeded the concentration in the outside solution very little, if at all. When the proportion of oxygen was increased to the proper point, and a suitably rapid flow of gas maintained, then bromine (and also potassium) was concentrated in the tissues in a most definite way. Other investigations now in progress on excised root systems of barley plants yield evidence of the same trend. It can be shown, in addition, that the metabolic condition prevailing in the plant previous to excision of the roots determines in part the degree to which accumulation of mineral elements can take place subsequent to excision. In other words, the absorbing power of the root system cannot be evaluated without a knowledge of the particular metabolic state in which it has been placed.

I have sketched in barest outline a few of the considerations which lead me to emphasize the point of view that the removal of electrolytes from a culture medium and their storage in the plant cell is a process in which the metabolic activities of the cell are inevitably concerned. Energy exchanges must be involved, the ultimate source of the energy being sunlight. Now this is by no means the view always taken by soil chemists and plant physiologists. Sometimes there seems to exist a fear that the adoption of such a point of view will lead to an undesirable "vitalistic" theory. In other instances, efforts are made to bring the explanation of the absorption of mineral elements by plant cells into line with the properties of some type of artificial cell. Some investigators emphasize the DONNAN equilibrium, so important in certain systems, but it is difficult to understand how this concept can possibly fit the observations which have just been cited. It is essential to keep in mind that even the simplest plant cell is an extraordinarily complex system in which energy exchanges are continuously occurring, and that these cell activities have an intimate relation to the absorption of mineral elements, as well as to other cell processes.

The mechanism involved in accumulation is not now understood, although several ingenious theories have been proposed which take into account many, but probably not all the facts. Whatever the mechanism of the cell may be, I think we can agree that what may be called the collodion bag concept of an absorbing plant cell is hopelessly inadequate. In making this statement, I trust it will not be thought that I am lacking in appreciation of the work of several very able investigators, who are elucidating the properties of simple membranes.

Perhaps these remarks will serve to make it clear that the accumulation of electrolytes by plant cells is not a question of simple permeability. It is possible to construct an artificial cell which is very permeable to potassium, but which cannot accumulate that element. The term "permeabil-

ity" has come to mean too much. Also, it is obviously not correct to state that a solute enters a plant cell until a state of diffusion equilibrium is reached. In living cells dynamic equilibria are involved. Still less can it be said that the entry of solutes is a process of osmosis. A more general aspect of the whole question can well be summarized by a quotation from a lecture on "Physical Chemistry in the Service of Biology" given last year by F. G. DONNAN. "Life, however, is not merely an affair of equilibrium and micro-structure, of oriented molecules and ions and delicately balanced micelles. Within and around this framework there exists a continual flux of physical and chemical action. The protoplasmic system operates by utilizing the free energy of its environment. It is a free energy converter, utilizing the free energy of oxygen plus convertible organic substances, of complex organic substances alone, and of sunlight. It is a physico-chemical machine, a dynamical or kinetic system. It is a truism of biology to say that the living organism cannot be considered apart from its environment. But this environment must possess some quality of non-equilibrium. It is the unbalanced environment which is the source of life and action, whilst the living organism is but the energy transformer, though one of peculiar and still mysterious character."

The view that the metabolic activities of the cell are of paramount importance in determining the accumulation of mineral elements by plant cells is not simply a scientific generalization. It has much practical significance to the investigator of soil problems. It means that special emphasis must be given to those soil and climatic conditions which favor root growth and activities. Soil aeration becomes of great concern, as does also the environment around the top of the plant, which may modify the supply of metabolites to the root system. The power of the soil to supply mineral elements to root cells will influence the synthesis of organic substances, which in turn will influence the continuing ability of the root cells to absorb mineral elements. Thus, especially with long-lived plants, either a normal or a vicious cycle may be established.

Let us assume now that we have the proper metabolic conditions for the accumulation of mineral elements by the plant cell. We are still faced by the great problem of the relation of the composition of the culture solution to the actually occurring accumulation. What are the effects of concentration and of ionic relations? Perhaps the first thought will be that we have at hand a great fund of information upon which to draw, in the enormously extensive literature on antagonism and permeability. This field of study has long been a major interest of plant and animal physiologists, and a few basic principles are agreed on. But closer examination discloses that the work on antagonism has nearly always dealt with very different prob-

lems and conditions than those in which we are now interested. You will recall that our objective is the understanding of plant growth under soil conditions, with special reference to crop plants. We are concerned with the gradual absorption and accumulation of certain chemical elements during the growth cycle of the plant. Experiments on antagonism and permeability are generally conducted with solutions of much greater concentration than those we are primarily discussing, and the time periods employed are often very short. Frequently the purpose is to utilize a decidedly toxic solution and then to determine how such toxicity may be overcome by suitable balancing of elements. Many investigators have employed the technique of plasmolysis which is obviously unsuited to the study of the relatively slow normal processes of absorption. Furthermore, in the majority of cases the antagonism demonstrated is concerned simply with cations. On the whole, it would seem that we must expect only very limited assistance from the investigations on antagonism in connection with the special problems now before us.

We do, however, have to take careful thought of the influence of one ion on the accumulation of another, whether or not the term "antagonism" is used. There is definite and extensive experimental evidence for the statement that importance attaches to each general type of ionic relation; namely, anion-anion, cation-cation, and cation-anion. It will be worth while to cite some specific illustrations. Referring again to the experiments on *Nitella* cells, we find that the interrelations of anions are noteworthy. Bromine ions and chlorine ions have reciprocal relations to each other and may undergo extensive interchange between the cell and the medium. One may retard the accumulation of the other. It is also possible to provide illustrations of direct agricultural significance. Under appropriate conditions of solution culture technique, in experiments with such plants as wheat or barley, it may be shown that there exists a reciprocal relation between nitrate and phosphate. By decreasing the concentration of phosphate in the culture medium at certain stages of growth, the plant may be caused to accumulate more nitrogen. Conversely, by decreasing the concentration of nitrate, plants sometimes may be caused to accumulate a very high content of phosphate. Interrelations between cations and anions are less easily demonstrated with complex solutions, but with simpler solutions it is feasible to obtain clear indications that the entry of cations may be facilitated by the presence of rapidly absorbable anions, such as nitrate. All these relations have interest to soil investigators, but such relations will of course not necessarily find expression under all soil or solution culture conditions. If marked deficiencies of several elements exist, other and more complicated situations arise.

The interrelations of cations are emphasized in the results of many types of plant experiments, including not only those in which artificial culture methods are employed, but also practical field experiments. If I may refer to our own investigations, merely for purposes of illustration, the interrelation between calcium, magnesium and potassium has been evidenced in a most consistent manner in many types of plant experiments we have had occasion to carry on during the past several years. As the potassium content of the culture medium is increased there is a very general and consistent tendency for the amounts of calcium and magnesium accumulated by plants to be decreased. Occasionally, under solution culture conditions, there is practically an equivalent substitution of bases. I should like to emphasize that these interrelations between bases are effective in very dilute solutions. A difference of a part or two of potassium in a million parts of solution may be of great consequence. The literature affords numerous indications that the interrelations of bases are also effective under many soil conditions, although the interpretation placed on results is not always the same as the one now advanced. German, English and French investigations could be cited, as well as investigations reported in this country.

It is well now to remark that caution is one of the safest attributes of the investigator of soil and plant relations. If I should make any broad generalization from experiments such as those which have just been cited, immediately I should be confronted with evidence obtained under other experimental conditions, and especially under diverse soil conditions, which could not be explained by anything contained in the preceding discussion. Under such circumstances all we can do is to take refuge in that word so precious to the biologist, "factors." The factors involved in plant growth under soil conditions are so entangled together, cause and effect so interwoven, that prediction is perilous. Not only the soil and the plant, but also the climate, must contribute factors to the extraordinarily involved equation, the solution of which would be necessary in order to obtain an adequate and precise explanation of the behavior of a given plant on a given soil at a given time. This does not mean that there should be a cessation of effort directed toward the elucidation of general principles, but it does mean that in any instance the operation of one set of processes may be masked by the operation of another set of processes.

If we could assume some principle of simple limiting factors our path would be smoother, but often such an assumption is not permissible. Consider, for example, the interrelation of light and potassium. Fertilization with this element may be much more effective in some seasons than in others. One writer in England has suggested that potash fertilization

may be the best insurance against a sunless summer. You will appreciate that we do not have sunless summers in California, but the general question has had some interest there, and opportunity has been afforded to observe the growth of plants under different conditions of light and of potassium supply. We may easily arrange an environment such that a large increase in plant growth may be obtained by doing either one of two things, increasing the light, or increasing concentration of potassium in the culture solution. This has been shown in greenhouse experiments, and recently a colleague has demonstrated the existence of these interrelations in the course of an investigation on the growth of plants under controlled conditions, with the use of artificial light. In one sense two limiting factors may operate at one and the same time, or perhaps more accurately, the efficiency of one factor is modified by the intensity of another factor. It will be immediately apparent to you how the problems of plant nutrition are complicated by considerations of this sort.

If you are willing to grant that various interrelations of ions do and necessarily must influence their absorption by plants, we cannot avoid the query whether there are certain special ratios between the various ions, best suited to the growth of a particular kind of plant. The attempts to discover "best solutions" have had value in stimulating research during the past fifteen years in the field of plant nutrition, even though the original objectives have suffered much alteration as the years have passed. Vitally important to such researches is the appraisal of plant variability and the statistical significance of any particular yield of plant material.

Probably we are now in a position to say with a good deal of assurance that there is no "best" solution for the growth of a particular plant, in so far as yield or the usual criteria of quality are concerned, and within the limits of error of any practical interest. Instead there are in general many solutions equally effective for plant production, varying to a greater or lesser extent in composition depending on the kind of plant, and on the climatic conditions. This is not at all the same as saying that different solutions will ever produce identical plants with reference to all details of organic and inorganic composition, or of metabolism, for this would probably not occur. I am referring to yield as indicated by weight and by the commonly observed general characteristics of the plant. There may be a "luxury" consumption of certain elements from many types of solutions, but this is a different matter.

These statements are of interest to soil investigators as well as to plant physiologists. With our present knowledge of soil solutions, it would be impossible to account for actually observed conditions in the field, if plants did not possess great powers of adaptation to solution conditions. Illustrations

tions of this fact have arisen in recent experiments with a group of California soils. In the course of these studies—over a three year period—tomato plants and barley plants have been grown in soils deficient in available potassium, fertilized in various ways. Within the limits of rather small experimental error for such experiments it was possible to obtain plants of the same appearance, rate of growth, and final weight, under the influence of soil solutions of highly divergent composition in respect to their content of potassium, calcium, magnesium, and other elements. On the other hand, the same group of experiments furnishes equally strong evidence that when concentrations of potassium in the soil solution are maintained sufficiently low an unbalanced condition in the plant ensues, accompanied by inhibition of growth or the appearance of disease. The range of adaptation is often a broad one but not indefinitely so.

Experiments of this character show that the ratios between certain mineral elements present in the plant, for example, calcium, magnesium and potassium, are profoundly modified when a marked disturbance of nutrition is manifest. Yet it would be difficult to prove that these modifications are the primary cause of the metabolic disturbance. Perhaps in the case mentioned we should emphasize rather the balance between potassium and certain organic constituents. If we ask why such a balance should be important, we find no answer to the question, since the mechanism by which potassium operates is still essentially unknown.

It is a natural step now to direct the discussion more definitely to the relations pertaining to concentration. The problems involved are difficult and elusive. The question to be answered is: What magnitudes of concentration of an element are adequate to maintain a suitable rate of delivery to the plant, if a given concentration of the element is maintained fairly constant in the culture solution surrounding the plant roots? For reasons which have already been given, no general answer could be expected to such a very broad question, but perhaps we can make some useful observations. First of all, it is essential to note that the work of several investigators is in general agreement on the point that extremely dilute solutions, at least of potassium and phosphate, may produce highly satisfactory growth of common agricultural plants. But it is obvious that critical zones of concentration exist. It needs no argument to show that concentrations may become so low that a plant may be unable to absorb in a unit of time an adequate supply of an essential element. It is also scarcely necessary to remind you that climatic conditions will alter the rate of supply required. Of great importance, likewise, is the kind of plant and the stage of its growth. The influence of one ion on the absorption of another has already been mentioned.

There is much investigation yet to be done on the relation of concentration to absorption and to plant growth. The objective should be the study of what has been called "supplying power," at different levels. Unfortunately the technique of such studies is laborious and expensive. Flowing culture solutions may be needed, or else some other procedure almost equally troublesome. The type of plant, the stage of growth, and the climatic environment all complicate the study of this question.

Any discussion of the absorption of mineral elements by plants must include some statement with reference to the relation between the intake of water and the intake of mineral elements. This is an old question, upon which different views have been expressed from time to time. A very simple experiment may be cited to illustrate one important phase of the relationship just mentioned. Young barley plants were transferred to a carefully measured volume of culture solution of known composition. The arrangements were such that water could escape only through the plants. After a period of about two days the residual culture solution was analyzed. It was found that the plants had effectively diluted the solution with respect to phosphate, potassium and nitrate, while sulphate was increased in concentration. In this particular experiment calcium happened to remain at almost its original concentration. Thus it is possible for ions to be absorbed more rapidly or less rapidly than water, or at the same rate. Sometimes attempts are made to compute the concentration of a soil solution from a knowledge of the total water transpired and of the amount of an element taken into the plant. It is clear that such computations are not well founded, since plants can bring about the dilution or concentration of a culture solution under some circumstances. Obviously all the conditions of metabolism within the plant, and the conditions of the environment surrounding the plant, will determine in any given instance the particular relation between absorption of water and that of any given ion.

Simple absorption experiments also throw light on other aspects of the selective action of plants. Some elements essential to the plant may be absorbed with great rapidity, but other elements not essential, except possibly in minute quantities, may likewise be removed from solution with great ease by plants. The predominance of potassium over calcium absorption is easily demonstrated with some types of plants, but with other types calcium may be absorbed as readily as potassium. Comparative rates of anion absorption vary with different plants and with different environments. All facts considered, there is no straightforward way of applying the physical chemical properties of ions in any entirely general or consistent manner. We do not know how to evaluate these properties in their relation to different protoplasmic systems, and to the varying

activities of such systems, although we may surmise the existence of logical relationships.

In these days it would be very unorthodox to discuss soil and plant relations without referring to hydrogen ion concentration, but within a space of fifteen years the literature has grown so vast in its extent that one hesitates to mention the question at all. Only one or two observations particularly pertinent to our present purpose will be ventured. I recall some early experiments with barley seedlings in California. With considerable surprise it was noted that the plants made better development in a definitely acid solution than in a slightly alkaline one. The impression gained from the literature of that time was to the effect that most agricultural plants found their most favorable environment in a slightly alkaline medium and were injured by acidity. It was a great gain to utilize physical chemical concepts in making measurements of the actual concentration or activity of hydrogen ions present in a culture solution. Yet I am unable to share the enthusiasm of those investigators who, by inference at least, assume the hydrogen ion concentration of the medium to be the almost exclusive determinant of plant distribution or adaptation. Save in the most extreme cases, it seems that hydrogen ion concentration should always be evaluated in relation to the concentration of other ions. For example, in recent years many investigators have called attention to the great importance of calcium ion concentration in this connection.

Concerning the relation of hydrogen ion concentration to absorption of other ions, we seldom have available experiments of an unambiguous nature. We cannot work over any large range of hydrogen ion concentrations and maintain all other properties of the solution the same, such as iron, phosphate and bicarbonate concentrations. There are some definite suggestions of the influence of hydrogen ion concentration on absorption of other ions, but they are not so clear cut and consistent as to warrant further discussion at this time.

Let us now speak a little more specifically of soil conditions. In 1911 CAMERON published a volume with the title "The Soil Solution." Certain very radical ideas, which had occasioned extensive controversy, were summarized in this volume. It is now well agreed that some of the conceptions of soil equilibria advanced were erroneous, and led to incorrect deductions. There is no longer any reason to doubt that the soil solutions of different soils may be of very different composition, or that the soil solution of any one soil may vary in composition as a result of seasonal changes, crop growth, or the application of fertilizers. Nevertheless, the controversy aroused probably had a beneficial result to soil science. A dynamic point of view of the soil was stressed, and many investigators were induced to

think of the soil as something very different from a storehouse of nutrient elements, needing to be inventoried, or as a more or less inert medium to which plant foods were to be added. A long period of intensive research on soil solutions has followed, in which various laboratories have participated, especially during recent years. I wish to touch on several of the leading features of these researches in their relation to what we have already been discussing.

Most of the early work dealing with soil solutions was conducted with the use of water extracts of one type or another. It was appreciated that such extracts could reflect only imperfectly the "soil solution," that is, the solution in the soil as it existed at some moisture content suitable for plant growth. Some studies were made in which soil solutions were separated from the soil by very high pressure, but these were limited in extent. A number of years ago a method was developed in Wisconsin, and later in California, by means of which the soil solution was displaced from moist soils by a supernatant column of water or of alcohol. Subsequently much intensive effort has been devoted to this method of obtaining soil solutions. It has been shown that according to certain chemical criteria solutions displaced from soils in this way may often be considered to be practically identical with the solution phase of the soil, as it exists at the moisture content at which the displacement was made. The arguments advanced need not be discussed on this occasion.

If a solution can be displaced from a soil with the characteristics just noted, have we then available an effective means of determining the composition of the plant's culture medium in the soil? This question has been presented in a very definite way in connection with researches of recent years on the phosphate nutrition of plants. It was suggested first by experiments made in Alabama that certain soils give displaced solutions with contents of phosphate entirely inadequate to account for the actual crop growth known to occur in these soils. The situation can be illustrated readily by an account of some later California experiments. Occasion arose to investigate a particular soil in which the phosphate present was extremely unavailable to many plants. The addition of suitable amounts of soluble phosphate enormously improved the physiological state of this soil, so that good growth of crops could be obtained. Yet solutions displaced from the fertilized soil did not give significantly higher concentrations of phosphate than those from the unfertilized soil. In all cases the values were extremely low. It was difficult to escape the conviction that the plant growth actually occurring in the fertilized soil could not have taken place if the displaced solution represented the real culture medium of the plant with respect to phosphate.

Are we to assume, therefore, that phosphate is not in fact absorbed from the soil solution, but rather that it is assimilated in some direct way from colloidal matter of the soil? This idea has been suggested, but it is not an inescapable deduction from the known facts. Certain essential considerations have not always received due emphasis, perhaps because of their very obviousness. Soils are heterogeneous and plants can deal with such systems in a heterogeneous manner, effecting a sort of physiological integration of many localized soil solutions. Laboratory procedures, on the contrary, deal with a mass of soil through methods which yield only composite solutions. Furthermore, in the laboratory, reactions such as those of fixation, may be facilitated by the very operations which the chemist must make, and which may have no counterpart in the soil as the plant grows in it, at least not in respect to rate. We must conclude then that the soil solution, no matter how prepared, cannot be assumed to be necessarily identical with the physiologically effective soil solution. If it is so assumed, then in certain circumstances an entirely erroneous idea may be gained of the solutions existing at the closely contacting surfaces of root membranes and soil particles. But it does not follow that a general soil solution theory of absorption is erroneous, merely because our methods of study are imperfect.

If no more were said on this point a wrong impression would be left. It has seemed necessary to draw your attention to some of the difficulties which may be met with in soil solution studies. As a matter of fact, difficulties of the same degree as those applying to phosphate have not as yet arisen in the study of concentrations of nitrate, calcium, magnesium, sulphate, nor even of potassium. With these ions the reflections of the physiologically effective solutions, although far from perfect, have been very serviceable. In general the magnitudes of concentration in the displaced soil solution have not been found to be radically different from those which are adequate for plant growth in a flowing culture solution. Even for phosphate this statement is true of many western soils. Notwithstanding the inherent difficulty of interpreting soil solution data, and the inconsistencies often arising, this method of investigating soils still remains one of the most important of tools, which can be employed with profit for a long time yet to come.

As soil solution researches have been continued, it has become more and more apparent that soil solutions should be studied in conjunction with the solid phase of the soil, for the nature of the solid components determines the power of the soil to keep the soil solution renewed as withdrawals by plant roots proceed. I shall mention one or two examples. The phenomenon of base exchange is now in the forefront of the discussions of soil chem-

ists. Much physiological interest attaches to the potassium combined in the base exchange complex. The experimental evidence shows that the potassium present in this form is in general available, when present above certain minimum amounts, varying with the soil. It can easily be assumed, and there are many results of experiments in favor of this assumption, that hydrogen ions produced by the activities of root cells easily displace a certain proportion of the potassium contained in the base exchange compound. The rapid intake of potassium by the plant continuously disturbs the chemical equilibrium and in a selective manner, which is not imitated in the laboratory. The potassium in the base exchange complex is, however, not always indispensable. The soil solution potassium may be maintained in some soils at adequate levels of concentration through the dissolving of other types of minerals. In fact, it is reasonable to postulate that there is involved the resultant equilibria between all of the various types of potassium compounds and the soil solution. These are ideas which cannot be expanded now, but the illustration may serve to emphasize the intimacy of the relation which should exist between studies on the solid and solution phases of the soil. If time were available much could be said also of the clarification which has come from base exchange studies with reference to acid and "alkali" soils, and the supplying power of the soil for calcium under these special soil conditions.

The relation between the solid and solution phases of the soil has a very practical agricultural aspect in the study of availability and the fixation of potassium and phosphate by the solid phase. Although among the earliest observed of soil phenomena it is doubtful whether the full import of such fixation is generally appreciated. It must be emphasized that fertilizers react chemically with soils. As has already been said, it is not merely a question of adding so much "plant food" to an inert medium. For example, we should inquire, in the case of deep-rooted plants, whether by fertilization we really change the soil solution as we desire in all the zones of root absorption. Even with shallow rooted plants the understanding of the degrees of fixation of phosphate and potassium with reference to availability to different types of plants is still entirely inadequate. We have to take account not only of the base exchange reactions, but also of other types of fixation. And the old questions concerning the importance of acid excretions by roots and of extent of absorbing root surface are not yet dismissed. It is clear that in these matters soil chemistry and plant physiology cannot be divorced, and that the practical art of soil management must have as its scientific basis a definite concept of the chemical and physiological interrelation between soil and plant. There are many times when the study of the plant itself will best forward an understanding of soil con-

ditions. It is true that most of the data of past years on inorganic plant composition have not carried us far, but experiments under suitably controlled conditions still offer much of promise.

I presume that before closing I am entitled to make a few general observations. I have spoken of past researches. What are the prospects for the future? We may expect that researches of the type already described will continue and will be greatly expanded. But we must acknowledge that the real reason we are so much interested in the mineral nutrition of plants is because of its relation to the synthesis of organic substances. We may hope and anticipate that the organic metabolism of the plant will excite more and more interest. There are indications in recent research that this movement is already well under way. Much will be learned from the further application of existing methods of research, but far more than this is required. The knowledge of the chemical nature of most plant constituents is entirely inadequate and consequently the methods used in following metabolic changes highly imperfect and incomplete. The plant physiologist will need to call to his service more men who are trained primarily as chemists or physicists. A few of the men now entering on their research activities will combine an excellent degree of training in both modern chemistry or physics as well as in biology, and from these much may be hoped in the future. Ordinarily it will be impossible to make an adequate advance in the phases of plant physiology of which I am speaking, without cooperative effort, not of the formal and official type, but rather of that informal type of cooperation which results from a common interest in a general objective by men of different specialization.

In addition to the intensive study of plant tissues by the methods of the organic chemist and the physical chemist, we may foresee an era in which great efforts will be made to grow plants under reproducible conditions, which cannot be done without control of light, temperature and humidity, as well as of the culture medium. As you know, such experiments have already been begun in Europe and in America. The difficulties of growing plants of any desired type satisfactorily under real control are very great, and not yet solved, but definite progress has been recorded with a few types of plants. It becomes possible to do exactly the same thing twice and those of you who are engaged in researches on the nutrition of plants will grant the cogency of this remark. A quantitative plant physiology in relation to mineral nutrition will develop gradually. From this and from the specialized chemical and physical research which I have just mentioned, we may hope for at least partial answers to numerous questions which now perplex us. I do not say complete answers, because as long as an investigator deals with living organisms he cannot hope ordinarily to achieve the

kind of solution of a problem which is often arrived at by the chemist or physicist, dealing with specially selected and simplified systems.

There is no compulsion on me to refer to the practical significance of research on soil and plant relations in an address of this character, but we cannot help recalling that practical interest will in a large measure dictate the support of research. Thus far there has been no adequate appreciation of the ultimate economic significance of plant physiological investigation in relation to soil problems. Its ramifications in the field of general agriculture are obvious, but of much wider scope than is always realized. Such research should be the vital foundation of the great fertilizer industry. Great sums are spent by this industry in practical tests and in publicity; relatively little on research dealing with basic principles. Agricultural experiment stations are lending important aid, but they have manifold demands on their resources. Yet I venture to predict that these physiological problems are going to receive ever increasing attention and support. If some speaker before this society in a not distant future shall elect to discuss the same field of investigation, I am confident that his report will be far more convincing and adequate than the one that I have been able to present this evening.

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INFLUENCE OF ACETIC, PROPIONIC, NORMAL BUTYRIC AND SULPHURIC ACIDS AND POTASSIUM ACETATE ON ELONGATION OF PRIMARY ROOTS OF SEED- LINGS OF WHITE LUPINE*

MARY COGGESHALL

(WITH TWELVE FIGURES)

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* Botanical contribution from the Johns Hopkins University, no. 114.

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Introduction

The influence of chemical compounds upon growth and development of organisms is of unfailing interest. The natural environment is continuously supplying the organism with innumerable water-soluble compounds, some of which are essential to nutrition and health while others are not essential. Any of them may be injurious if absorbed too rapidly or in too large an amount. Their absorption rates depend in large measure on their concentrations in the surroundings. Many substances that have markedly injurious effect when supplied to an organism in one concentration range have been found to stimulate or accelerate growth and other activities when supplied in a lower concentration range. Too high concentrations of usually essential compounds are harmful but physiological processes eventually become retarded or upset when the essential compounds are inadequately supplied. Large numbers of apparently unessential chemical compounds are commonly present in organisms and in their surroundings without producing any physiological effect, either of retardation or stimulation, but any of these may have a marked effect if its concentration is sufficiently increased. Furthermore, the kind and amount of influence exerted by a specified compound at a specified concentration on a given organism in a given stage of development or physiological state is itself greatly influenced by the prevailing concentrations of other compounds. The favorable or unfavorable influence of a certain compound at a specified concentration in the environment may be greatly altered in many instances without any change in the concentration of the compound in question, through suitable alterations in the concentrations of other compounds. Finally, different kinds of organisms or individuals of the same kind in unlike developmental phases or with different degrees of vigor may be affected very differently by one and the same set of chemical concentrations in the surroundings.

Various aspects of the chemical relations of organisms have been studied by many investigators. The whole science of pharmacology and the continually expanding study of antiseptics and their applications deal with these chemical relations. Applied plant pathology and the control of insect pests offer innumerable problems of differential toxicity. From the standpoint of agronomy and horticulture the chemical relations between higher plants and the chemical compounds in the soil solution have received a very large amount of attention. The study of the mineral nutrition of plants deals wholly with chemical relations and chemical influences and upon its findings since the time of LIEBIG have been built up modern fertilizer practice and the great fertilizer industries. Research in this general field continually results in new knowledge and leads to new applications,

while practical investigations result in scientific as well as practical advance. Some of the most fundamental theoretical conceptions of physiology are largely based on knowledge of chemical retardation and stimulation gained through experiment; for example permeability, enzyme action, narcosis, anaesthesia, the breaking of physiological dormancy and many others.

The nature of the problems concerning chemical influence on organisms is such that any single experimental investigation must necessarily be confined to a small number of compounds if many different concentrations are studied or else to few concentrations of each compound if many compounds are dealt with. Also, it is usually feasible for an investigator to work with only one kind of plant or animal or with but a few different forms, and with only one or a very few different developmental or physiological phases of any organism. Many plant forms have been studied in this connection by different workers, ranging from bacteria, fungi and algae to such plants as maize and sunflower. For the higher plants soil cultures and solution cultures have been employed to bring out the various influences exerted by solutes in the aqueous solution about the plant roots. Only rather recently have the influences exerted by gaseous compounds in the air about plant parts been subjected to extensive study, especially in connection with the ripening of fruits and other plant products in storage. The substances already mentioned in the literature of chemical influence represent almost the entire range of chemical compounds, from the simplest inorganic salts and carbon compounds like carbon monoxide, carbon dioxide and ethylene to the most complex substances with which chemical science struggles, like proteins, alkaloids and glucosides.

A vast literature is available concerning the influence of inorganic compounds on plants. Some of these compounds supply essential nutritive elements, as nitrates, phosphates and sulphates of potassium, calcium and magnesium. These and others are commonly present in the soil solution, sometimes in highly injurious concentrations, and still others have been studied primarily to throw light on the general theory of toxicity and stimulation. In Szűcs's article (26) may be found a discussion of antagonistic ion absorption in plants. A general summary of our knowledge of inorganic plant poisons has been made available by WINIFRED BRENCHLEY (1).

Organic compounds have received much attention, especially in connection with applied science, as in medicine, in the search for new antiseptics and in the field of stimulants, narcotics and anaesthetics. Many carbon compounds have been studied to some extent in connection with the problems of nutrition and of biochemistry in general. Substances that usually

occur in the plant or animal body or in natural environments have attracted much attention at the hands of experimenters. Studies have been made on some of the simpler organic compounds as they may influence bacteria, fungi or lower animal forms. Much fundamental research on lower animals has been devoted to the lower fatty acids and related substances, especially with reference to permeability and cell division.

A few recent papers may be mentioned. MARY E. COLLETT (5) studied the influence of organic and inorganic acids on *Paramoecium* and *Euplotes*. CROZIER (6) studied the permeability of the cells of *Chromodoris zebra* with reference to several aliphatic acids. H. W. SMITH (25) studied the influence of these acids on cell division in eggs of *Echinarachnius*. LUCKE and McCUTCHEON (17), working with *Arbacia* eggs, related the rate of penetration of salts of organic acids to valency and other molecular characteristics.

Much attention has been given to the toxicity of fatty acids in relation to bacteria and fungi (KRÖNIG and PAUL, 15; TAYLOR, 27.). J. F. CLARK (3) studied toxicity with reference to some filamentous fungi, finding, among other things, that acetic acid was more toxic than the common mineral acids. DUNN (8), working with the fungus *Sclerotinia cinerea*, thought hydrogen-ion concentration was the main factor determining the toxicity of the common mineral acids but that it was of only secondary importance in determining toxic action due to the simple fatty acids. UPPAL (32) studied the influence of various organic acids on the germination of spores of *Phytophthora colocasiae*.

Some organic acids have been studied with regard to their influence on the growth of higher plants. KAHLENBERG and TRUE (14), HEALD (10), KAHLENBERG and AUSTIN (13), TRUE (30) and CAMERON and BREAZEALE (2) experimented with white lupine, pea, maize, wheat, clover, etc. DACHNOWSKI (7) studied the influence of organic acids on the rate of transpiration of tomato plants. MACHT and his co-workers have employed root elongation in young seedlings of white lupine as an indicator of the relative toxicities of many organic compounds (MACHT and MARGUERITE B. LIVINGSTON, 20; MACHT and HARRIET P. LEACH, 19) and biological preparations (MACHT and DOROTHY LUBIN, 21; MACHT and W. T. ANDERSON, 18). MACHT has suggested the term "phytopharmacology" to mean the study of toxicities or other influences by means of plant organisms as indicators. EISENMENGER (9) studied the toxic effects of a number of aliphatic alcohols in nutrient solution, on the rate of root elongation of soy-bean seedlings.

In many studies concerning the influences of organic compounds on plants only a few different concentrations of each of the substances in ques-

tion have been tested and attention has been chiefly confined to lethal concentrations. Comparatively little attention has usually been given to the requisite standardization of the plant material employed or to the background conditions of the experiments. For these and related reasons and because most investigators in this field have chosen plant material markedly different from that used by others, very little progress has thus far been made toward any general understanding of even the simplest organic compounds in their relation to plants. Systematic study in this field has hardly begun and suitable technique for it remains mostly to be developed. The obvious necessities are: (1) That the plant material used should be as nearly the same as possible for the beginning of all comparable tests and should be so described as to permit repetitions of the experiments. (2) That all comparable experiments should be performed on material in the same developmental phase or stage of maturity—preferably a phase in which the rate of the physiological process to be measured does not automatically alter too much, in the healthy material used for controls, within the experiment period, or alters only in ways that may be satisfactorily taken into account in comparing the numerical results. (3) That the duration factor (lengths of observation intervals and exposure periods) should be given just as serious consideration as is accorded to any other influential feature. (4) That the background conditions of the environment for all comparable tests should be just as adequately controlled, or at least just as thoroughly specified, as are the experimental variables themselves, repetition of otherwise fairly satisfactory experiments not infrequently being rendered impossible because background conditions have not been suitably defined. (6) That the experimental variables (the compounds to be tested and their concentrations) should be adequately specified. (7) Finally, that the effects or responses of the plant material to the various treatments or environmental complexes should be measured with adequate precision and should be recorded in suitable terms for convenient comparison. The last two desiderata are commonly well cared for in experimentation but at least some of the others are apt to receive but scant attention. It is of course impossible as yet to fulfill all these requirements for ideal experimentation but it is surely desirable now to go as far toward the ideal as may be permitted by circumstances and facilities and by the nature of the research in question.

The study reported in this paper was largely an attempt to develop a fairly consistent and feasible technique for studying the concentration relations of chemical compounds to the elongation of the primary roots of white lupine seedlings. All the seedlings used were very nearly alike, having been grown from selected seeds under standard conditions. Their primary

roots were of nearly the same length and were in that developmental phase in which the rate of their elongation is automatically maintained for a long time with a maintained environment favorable to health. The treatment or exposure period, during which a chemical compound was present in the solutions around the roots, was of such length that control roots in standard nutrient solution always showed a definite increment of elongation in that period. The external background conditions were specified in terms of a standard nutrient solution, a maintained temperature and a standard experimental procedure. The three simplest aliphatic acids, the potassium salt of one of them and one mineral acid were the compounds tested, with a large number of concentrations of each compound. In each case the concentrations tested were so chosen that the entire concentration range from zero to the concentration that inhibited root growth in the treatment period was well represented. The results consequently furnish a picture of the manner in which the influence of each compound depended on its concentration.

This experimentation was carried on in the Laboratory of Plant Physiology of the Johns Hopkins University, with advice and guidance from the director of that Laboratory, Professor BURTON E. LIVINGSTON. The writer wishes to express her thanks to Professor LIVINGSTON for facilities and equipment as well as for many helpful suggestions concerning procedure and technique, and especially for help in the interpretation of results and in the preparation of this paper.

Methods and procedure

THE EXPERIMENT ROOM

All the experiments of this study were performed in the basement dark room of the Johns Hopkins Laboratory of Plant Physiology. There was some movement of air around the frame of the shuttered window and through the three-door labyrinth but no light entered the room. It was always dark excepting for a period of about three hours each day, when a 50-watt electric light was in operation, for observation and manipulation. The air temperature of this room remained nearly constant for long periods of time but when suitably placed thermographs showed lowering temperature an electric heater was brought into operation. Thus the air around the cultures was maintained between 18.8° and 21.0° , but the fluctuation for any single experiment was not as great as is indicated by these limits. A standardized white spherical atmometer situated near the cultures lost from about 13 ml. to about 20 ml. per day, which indicates low, rather uniform evaporation intensity.

PREPARATION OF SEEDLINGS

SEED GERMINATION.—The plant material used was seedlings of white lupine (*Lupinus albus* L.) with primary roots about 30 mm. long. These had all been grown from the same lot of seed, supplied by the Vaughan Seed Store, in Chicago, and stored in the experiment room. The seeds of this lot were very uniform in color, form and size—about 8 mm. in diameter and 3 mm. thick—and showed a germination percentage of 98 for the conditions employed. There was no evidence of any seed deterioration throughout the period of the study.

For each set of experiments, requiring 90 or 105 selected seedlings, about 400 seeds were soaked 24 hr. in tap water at a temperature of $20 \pm 2^\circ$ and were then planted in the germinators. These were cylindrical glass pans 22 cm. in diameter and 8 cm. high, containing washed quartz sand of medium fineness moistened with 5 ml. of standard nutrient solution for each 100 ml. of sand. This medium had a volumetric water-holding power of 29.6 per cent., according to the HILGARD test (12) with a 1-cm. column. At the beginning of the study and after each period of use the sand and the germinator pans were thoroughly washed with distilled water and allowed to drain. The sand was spread out on paper in the greenhouse, where it became air dry and ready for use.

Sand, moistened with nutrient solution, was rather firmly packed in the germinator pan to a depth of about 5.5 cm. Approximately 175 soaked seeds were pressed, micropyle down, half way into the smoothed surface, after which air-dry sand was added to fill the pan level with the top. At the end of a germination period of about 48 hr. the dry sand was poured off and the seedlings were lifted by means of bone-tipped forceps applied to the cotyledons and all apparently unusual seedlings were discarded.

Each of the selected seedlings from the sand culture was rinsed in standard nutrient solution and its root length was measured before it was placed in the preliminary solution culture. For any set of experiments the root lengths were all alike within plus or minus 2 mm. and the whole range of root lengths at this stage was only from 15 mm. to 22mm. Throughout these operations the roots were kept pointing downward and were not allowed to come in contact with the 15-cm. celluloid metric scale used in their measurement. About 35 acceptable seedlings with straight roots of the required length were obtained from each 100 seeds originally planted.

PRELIMINARY SOLUTION CULTURES.—For the preliminary solution cultures cylindrical stone-ware jars ("butter crocks") 30 cm. in diameter and 17 cm. deep were used as containers, each with a multiperforate circular cover of reinforced paraffin about 8 mm. thick. This paraffin plate was

prepared from quarter-inch galvanized iron wire netting by filling and heavily coating with paraffin. The perforations were made with a cork borer. Each plate bore about 200 seedlings, identified by India-ink numerals on the paraffin. The plantlets were supported by their cotyledons, each root and hypocotyl extending downward through one of the perforations and dipping into the solution to a depth of at least 8 mm. Each jar contained enough standard nutrient solution to bring the liquid surface nearly up to the plate. The seedlings remained in preliminary culture, where root elongation proceeded at a mean rate of somewhat over 1 mm. per hour until the roots were about 30 mm. long and the hypocotyls about 10 mm. They were then removed, measured, recorded and placed in the experiment tubes for the treatment period. At this transfer only those seedlings were used which had straight roots 30 ± 2 mm. long. About 25 of these standard seedlings were thus obtained from each hundred seeds originally planted.

In measuring for this selection, as well as for subsequent growth rates, only the roots were considered. The hypocotyls attained a length of about 10 mm. in the preliminary culture, as has been said, and their subsequent elongation was practically negligible until the roots had attained a length of about 70 mm. Measurements were from the root tip to the easily visible ring or collet that marks the junction of root and hypocotyl in seedlings of this species. Because the collet is somewhat irregular, all measurements of each particular root were made on the same side, orientation being secured by reference to the plane of the cotyledons. Throughout the study the maximum error of measurement was not more than 1 mm.

THE STANDARD NUTRIENT SOLUTION

The nutrient solution used as standard throughout this study was of SHIVE's (24) 3-salt type. It was prepared with distilled water from a Barnstead still and "C. P." crystalline salts, the latter supplied by the J. T. Baker Chemical Co., of Phillipsburg, New Jersey. Each liter of the standard solution contained 0.820 g. (0.0050 mol.) of $\text{Ca}(\text{NO}_3)_2$, 0.9395 g. (0.0069 mol.) of KH_2PO_4 , and 1.128 g. (0.0094 mol.) of MgSO_4 . The possible error for each salt was not over 0.6 per cent. in any instance. No iron was added. This solution had been calculated to have equal osmotic proportions of the three salts and a total osmotic value of about one atmosphere at 20° . It produced very satisfactory growth in the seedlings of this study but no attempt was made to find an optimal solution and it of course remains possible that some other combination of these or other salts might have produced even more vigorous root growth. Several preliminary experiments showed that the total concentration of this solution might be as

much as 25 per cent. higher or lower without significant alteration in root elongation.

An 18-l. lot of solution of each salt lasted throughout the entire study. The three single-salt stock solutions were stored in as many 5-gallon bottles in the experiment room. Each bottle had a rubber stopper bearing an air inlet, guarded by a calcium-chloride tube, and a siphon for removal of solution. The siphon was provided with a bit of rubber tubing bearing a Mohr pinch cock and a short glass tip.

The molar concentrations of the single-salt solutions, as ascertained by gravimetric analysis (TREADWELL and HALL, 28) were as follows: 0.500 mol. KH_2PO_4 , 0.532 mol. $\text{Ca}(\text{NO}_3)_2$ and 1.005 mol. MgSO_4 . The standard nutrient solution was prepared volumetrically from these single-salt solutions, usually 18 l. being made up at weekly intervals, or more often. It was stored like the single-salt solutions in the experiment room.

THE EXPERIMENT SOLUTIONS

EXPERIMENTAL VARIABLES AND BACKGROUND CONDITIONS.—By means of specified experiment solutions the physiological effects of the five compounds studied were ascertained by tests. These effects were of course measured in terms of the root elongation of standard seedlings. In the main part of the study the effect of each experiment solution was measured by comparing the amount of root elongation that occurred in it with the amount that occurred at the same time in standard nutrient solution without any added compound. In a single series of experiments distilled water was used instead of standard nutrient solution and the control medium for these was of course distilled water without any added substances at all. All experiment solutions in any series were qualitatively alike at the start for all tests but differed with regard to the concentration of the single added compound. The six series of experiment solutions are set forth below, with the number of different solutions in each series.

Series 1. Standard nutrient solution *plus* acetic acid at various concentrations. 35 solutions.

Series 1 A. Aqueous solutions of acetic acid. 19 solutions.

Series 2. Standard nutrient solution *plus* propionic acid at various concentrations. 28 solutions.

Series 3. Standard nutrient solution *plus* normal butyric acid at various concentrations. 28 solutions.

Series 4. Standard nutrient solution *plus* sulphuric acid at various concentrations. 18 solutions.

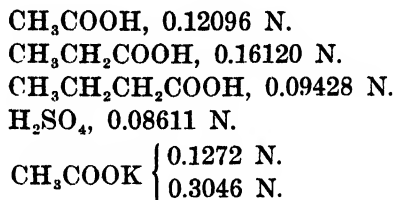
Series 5. Standard nutrient solution *plus* potassium acetate at various concentrations. 19 solutions.

In each series of experiments there was just one experimental variable, the concentration of the added compound, and all other influential conditions were approximately alike for the beginning of all tests in that series. When different series of the main group (Series 1, 2, 3, 4, 5) are compared two experimental variables have to be considered, the nature of the added compounds and their respective concentrations. When the series based on distilled water (1 A) is compared with any other series, the complex of the three nutrient salts is of course an experimental variable. Excepting the experimental variables and the process rate that was to be measured (elongation of primary root), all variables were approximately alike at the beginning of all tests; that is, they are to be regarded as constants for any series and for all series. These constitute the experimental background; they were either maintained within limits of fluctuation set by the general technique or else they altered during the tests in ways determined by the interaction of seedling and solution. Among the approximately maintained background conditions of the environment were temperature, light, evaporational intensity and oxygen supply. The internal characteristics of the standard seedlings were approximately alike at the start of all tests but they naturally altered with time and in accord with the influences exerted by the several experiment solutions.

It should be mentioned that the experiment solutions all altered while in contact with the roots. A liquid medium surrounding a living root changes in many ways, through the absorption of molecules and ions by the root and through the escape of molecules and ions from the root. To avoid this troublesome consideration it would have been necessary to arrange for the experiment solutions to flow continuously around and past the roots at adequate rates. This aspect of the technique of physiological experimentation has been emphasized by TRELEASE and LIVINGSTON (29) and some later writers, but its introduction would necessarily narrow the field of any experimental study. This difficulty is sometimes avoided to some extent by renewing the experiment solutions at intervals, if the experiment periods are of suitable length, but other troublesome considerations are thereby introduced.

PREPARATION OF THE EXPERIMENT SOLUTIONS.—The experiment solutions were prepared from standard nutrient solution or distilled water and simple stock solutions of the five chemical compounds whose influences on root elongation were to be studied. The acetic acid (CH_3COOH) used was Merck's "C. P.," 99.5 per cent. The propionic acid ($\text{CH}_3\text{CH}_2\text{COOH}$) was from the Eastman Kodak Co., with boiling point $140^\circ\text{--}142^\circ$. The normal butyric acid ($\text{CH}_3\text{CH}_2\text{CH}_2\text{COOH}$) was from the same source, with boiling point $161^\circ\text{--}163^\circ$. The sulphuric acid (H_2SO_4) and the potassium

acetate (CH_3COOK) were from the J. T. Baker Chemical Co., the former being "C. P.," 93.5–95.9 per cent. and the latter "C. P.," Baker's analyzed. The stock solutions had the following concentrations, as ascertained by titration:—



These were made up with distilled water and were stored in glass bottles in the experiment room.

The experiment solutions based on standard nutrient solution were made volumetrically, by diluting the proper simple stock solution with standard nutrient solution to the required degree. For the lower concentrations of the added compounds this was accomplished by several steps. The corresponding dilution of the nutrient solution was considered as negligible; for the most concentrated experiment solutions this dilution never amounted to more than 7 per cent. No experiment solution containing the nutrient salts was ever stored longer than one week.

The experiment solutions based on distilled water without nutrient salts were prepared from the simple stock solutions just as the others were, excepting that water was used instead of standard nutrient solution.

The actual concentrations of the added compound in each series of experiment solutions are shown in tables I–VI, expressed in terms of millionths normal. Thus 2,560 millionths normal means the same as 0.002560 N. Of course a solution so designated has 2,560 millionths of a hydrogen equivalent of the specified solute in each liter of solution. Normal and molar values are identical for the three organic acids and potassium acetate, but a normal-concentration value is numerically twice as great as the corresponding molar-concentration value in the case of sulphuric acid.

HYDROGEN-ION CONCENTRATION OF THE SOLUTIONS.—Approximate hydrogen-ion concentrations of representative samples of each series of experiment solutions containing the nutrient salts were determined by the colorimetric method of W. M. CLARK (4, p. 38). No buffer was added since all these solutions contained KH_2PO_4 at a concentration of about 0.0023 normal. The results, expressed in terms of the pH scale, are plotted in figure 1 with graphs showing the general trends indicated by the points.

From this figure it may be seen that the pH value for the standard nutrient solution was 4.4 and that all the solutions of every series were

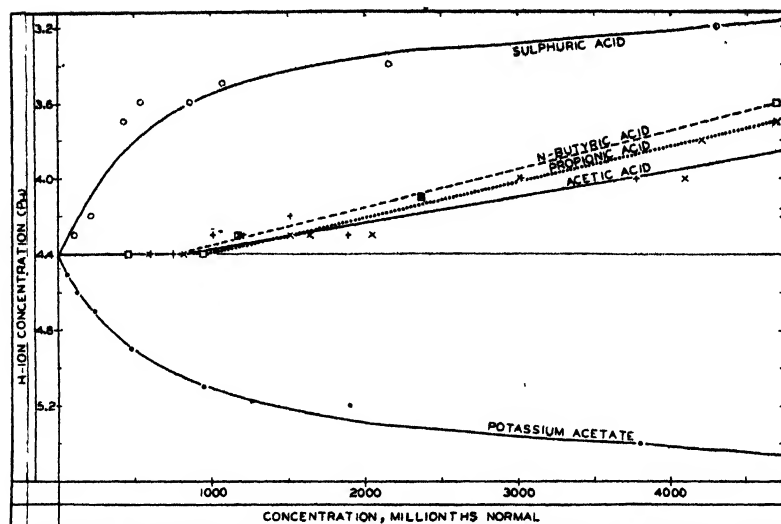


FIG. 1. Graphs showing approximate relations of concentration of added compound to pH value in each of the five series of modified nutrient solutions.

markedly acid. Of the five added compounds only potassium acetate produced solutions of pH values above 4.4. The addition of that salt, even in very small concentration, raised the pH value markedly. As would be expected from its chemical nature, sulphuric acid produced a marked lowering of the pH values of the nutrient solutions to which it was added, even when present in very low concentration. With progressively greater concentrations potassium acetate and sulphuric acid were less effective in altering the hydrogen-ion concentration of the nutrient solution to which they were added. It is interesting to note that these two graphs are nearly symmetrical about the horizontal line for 4.4, the graph for sulphuric acid lying above that line and the one for the acetate below.

The approximate graphs for the three organic acids appear in figure 1 as straight lines sloping upward to the right of their points of intersection with the horizontal line for the pH value of 4.4. These points are all far from the y axis, which means that a considerable concentration of any one of the three organic acids was required to lower the pH value below 4.4 sufficiently to be detected by the test method used. This critical concentration value was found to be about 775 millionths normal for butyric acid, about 825 millionths normal for acetic acid and about 950 millionths normal for propionic acid. But the precision of these colorimetric measurements is not high enough to warrant much emphasis on the exact order of these critical values.

THE EXPERIMENT PERIODS AND THE INDICES OF ROOT ELONGATION

GENERAL PLAN OF EXPERIMENTATION.—Measured seedlings were allowed to remain in the experiment solutions for a treatment period of about 20 hr., after which their roots were again measured. The seedlings were then rinsed in standard nutrient solution and returned to the preliminary culture from which they had been taken. They remained there for a recovery period of 10 hr. Measurements and observations made in this period showed the after effects of the preceding treatment with the experiment solutions. For the treatment period each seedling occupied a separate test tube with about 27 ml. of experiment solution but for the recovery period all of the seedlings in any experiment had their roots in the same large jar of standard nutrient solution. All experiments began with measured standard seedlings grown as described above, each experiment comprising 6 or 7 units of 15 seedlings each, all in any unit being treated alike. There was a control unit in each experiment, consisting of 15 seedlings in as many tubes of standard nutrient solution for the treatment period. An additional control of 15 seedlings in as many tubes of distilled water was used for the experiments on solutions without the nutrient salts. The seedlings of both types of control were returned to the nutrient solution in the preliminary-culture jar for the recovery period.

The average increment of root elongation was secured for each unit, for the treatment period and for the recovery period. These averages constitute the primary numerical results of this study. Each was finally expressed as a percentage of the average from the corresponding control unit for the same period.

THE TREATMENT PERIOD.—In the treatment period each plantlet occupied a separate rimless test tube of "Pyrex chemically resistant" glass, 15 mm. in diameter inside and 150 mm. long. Each tube had a paraffin-impregnated cork 17 mm. long, of the common taper form, with a central hole 5 mm. in diameter. The corks fitted the tubes so tightly that only about 2 mm. extended inside. Before the beginning of each experiment, tubes and corks were thoroughly washed with distilled water and dried by draining. When a change was to be made in the kind of experiment solution the tubes were washed with cleaning mixture (concentrated H_2SO_4 saturated with $\text{K}_2\text{Cr}_2\text{O}_7$). In use, the tubes were held in rectangular wire racks holding 40 tubes each. Instead of tubes or stoppers being marked the tubes were identified by their positions in the rack. A thermograph on the shelf bearing the racks showed a maintained temperature of $20 \pm 1^\circ$.

In setting up an experiment, each of the 15 tubes in each unit received about 27 ml. of the requisite solution and a cork was inserted. As the seedlings from the preliminary culture were measured and recorded each satis-

factory one was mounted in a tube of the requisite solution, after being rinsed in a beaker of the same solution. The root was inserted through the cork, on the top of which the cotyledons rested. At that time the roots extended about 17 mm. into the solution, the free surface of which was approximately 3 mm. below the top of the tube, or about 1 mm. below the bottom of the cork.

The treatment period for each experiment lasted until the seedling roots of the nutrient-solution control unit had increased in length by about 26 mm.; that is, until the roots of that control unit had a mean length of 56 mm. The length of the treatment period varied somewhat from one experiment to another because it was measured in terms of root growth of these control seedlings. Its actual duration was from 18 to 23 hrs. The variations may be considered as due to slight differences among the experiments with regard to prevailing influential conditions.

The preliminary measurements of the control roots in standard nutrient solution, for ascertaining just when treatment would be discontinued, were made by observation through the walls of the tubes, without removing the plantlets, and they were consequently less precise than the subsequent regular measurements at the end of the treatment period, for which the seedlings were of course removed.

At the close of the treatment period every seedling was removed from its tube and its root length was measured in the regular way. For each unit of the experiment in question there were thus obtained fifteen individual elongation increments for the treatment period. The average increment for this period was then computed for each unit and each average was expressed as a percentage of the control average for the same experiment.

The percentage values thus secured are taken as numerical indices of root elongation in the treatment period and those derived from different series of experiments, as well as from different units of the same series, are comparable for all cases based on the same kind of control solution. The final phrase of the last statement is important because distilled water was the control medium for the tests made with solutions that did not contain the three nutrient salts while standard nutrient solution was the control medium for the other tests. These considerations may be illustrated by the following examples of typical measurements and computations for the treatment period. See page 404.

It is seen that the average elongation for unit B is expressed as a percentage of the corresponding average for control unit A, while the percentage index for unit D is based on the corresponding average for control unit C. These data are from one experiment for units A and B and from

Seedling no. in experiment unit of 15 seedlings	Root elongation in treatment period			
	Unit A, standard nutrient solution	Unit B, standard solu- tion with addi- tion of 1129 millionths normal $\text{CH}_3 \cdot \text{COOH}$	Unit C, distilled water	Unit D, 605 millionths normal $\text{CH}_3 \cdot \text{COOH}$ without nutrient salts
	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>
1	22	17	8	4
2	25	20	7	6
3	27	19	6	2
4	25	13	7	2
5	25	16	5	3
6	24	17	7	3
7	28	14	6	3
8	24	21	9	4
9	26	17	3	3
10	28	12	5	4
11	28	19	7	6
12	28	14	3	4
13	23	21	4	2
14	19	16	7	5
15	21	18	6	3
Average for unit	24.8	16.9	6.0	3.6
Percentage index	100.0	68.3	100.0	60.3

another experiment for units C and D. All percentage indices for the treatment period based on controls with standard nutrient solution are comparable throughout the whole range of this study. This is also true of all percentage indices based on controls with distilled water, but the two groups are to be kept distinct.

THE RECOVERY PERIOD.—At the close of the treatment period each measured seedling was rinsed in standard nutrient solution and then returned to the paraffin plate on the preliminary-culture jar from which it had been removed for treatment. The solution in the jar had not been changed. After an interval of about 10 hrs. all roots were again measured and percentage indices of root elongation were derived in the same way as for the treatment period. These furnish a second series of growth indices

for each compound studied. Of course they refer to the after effects occurring in the 10-hr. interval after the treatments had been discontinued. The seedlings were left on the paraffin plate for several days and further observations were made, but branching soon occurred in the upper region of many injured roots and consistent measurements were then difficult or impossible. Only the first 10 hr. following the close of the treatment period will be considered in this report.

ROOT ELONGATION IN THE CONTROL CULTURES

THE MARCH OF ROOT ELONGATION IN CONTROLS WITH STANDARD NUTRIENT SOLUTION AT 20°.—The rate at which an organ enlarges usually alters as development proceeds, even when the environmental influences are all maintained. For organs of limited growth, such as these primary roots, elongation under favorable external conditions is slow at first, then increases to a relatively rapid rate, which may be maintained while a large increment is added, and finally decreases as maturity is approached. This grand march of the growth rate is controlled by internal changes when external conditions do not alter in the experiment period and it may be modified in any phase by stimulating or retarding influences acting from the outside. In the lupine roots used in this study, when grown in standard nutrient solution by means of the technique described above for preliminary cultures and nutrient-solution controls, elongation ceased or became very slow after a rather definite length had been attained, and further root growth then took the form of development of lateral branches.

Since the results of most of these experiments were to be expressed with reference to control cultures in standard nutrient solution, it was important to know how the rate of root elongation changed as development advanced when the roots were kept in standard nutrient solution and the other conditions were those of the regular nutrient-solution controls. Three special experiments and two of the regular control units were employed to give information in this respect. These tests were performed at different times between October and February. Seedlings were removed from the germinator 26 or more hours after planting, being then measured and immediately placed in experiment tubes of standard nutrient solution, where they remained for a period of about 150 hr. The temperature was $20 \pm 1^\circ$. In this period measurements of root length were made at intervals, in the regular way. The resulting data are shown graphically in figure 2, where the ages of the seedlings (reckoned from the time of planting the seeds) are plotted as abscissas and the root lengths are ordinates. Each of the different symbols represents a separate experiment.

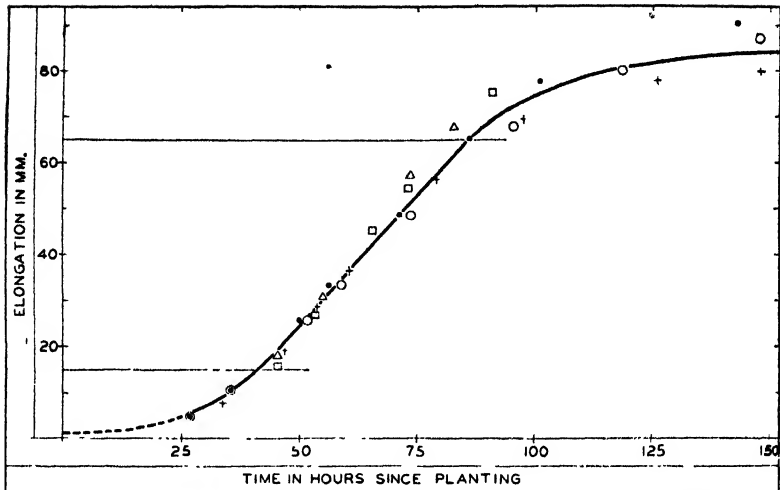


FIG. 2. Graph showing grand march of primary-root elongation in *standard nutrient solution*; the several symbols represent different experiments.

All the points taken together form a remarkably homogeneous and consistent series. The average march of root length with respect to time is approximated by the curve. The growth rate is shown to have increased until about the fortieth hour, when the average root length was about 15 mm., after which the average rate was maintained (at a little over 1 mm. per hour) for about 45 hr., until the average length was about 65 mm. The rate then decreased and at the end of about 150 hr. it was only about 0.2 mm. per hour, when the root was about 85 mm. long.

This graph is similar to other curves for the grand period of growth in plants. In connection with the root-elongation indices to be considered below, the maintained rate between root lengths of 15 mm. and 65 mm. is specially interesting. This range of root lengths represents a phase of rapid enlargement at a nearly constant rate. In this phase enlargement was practically confined to the primary root. The cotyledons remained close together and without marked change, elongation of the hypocotyl was very slow and secondary roots had not yet begun to appear. At the end of this phase the hypocotyl began to show rapid elongation.

Most of the experimentation of this study is referred to this developmental phase of plantlets in the nutrient-solution controls. According to the regular procedure the roots of the standard seedlings were about 30 mm. long and those in the nutrient-solution controls became about 56 mm. long in the treatment period and about 66 mm. long in the recovery period.

INFLUENCES OF SLIGHT TEMPERATURE FLUCTUATIONS ON ROOT ELONGATION IN NUTRIENT-SOLUTION CONTROLS.—The temperature at which the experi-

ments of this study were conducted fluctuated between extremes of 18.8° and 21.0°, as has been indicated, and the results to be presented farther on consequently refer to a maintained temperature of about 20°. The following data seem to show that the temperature deviations may generally be neglected. These data refer to regular control units with standard nutrient solution, for the treatment period of about 20 hr.

Maintained temperature	Length of period	Mean hourly rate of root elongation	Maintained temperature	Length of period	Mean hourly rate of root elongation
<i>deg. C.</i>	<i>hours</i>	<i>mm.</i>	<i>deg. C.</i>	<i>hours</i>	<i>mm.</i>
18.8	19.2	1.29			
19.0	21.1	1.24	20.2	{ 19.8	1.23
19.5	19.4	1.24		{ 19.8	1.26
				{ 19.7	1.31
19.8	{ 20.2	1.34	20.5	{ 20.3	1.37
	{ 20.0	1.28		{ 20.2	1.24
	{ 20.0	1.29	20.8	20.4	1.38
	{ 18.6	1.31		{ 23.1	1.18
	{ 24.2	1.11	21.0	{ 22.1	1.34
	{ 22.3	1.18		{ 20.4	1.27
	{ 19.9	1.26		{ 20.4	1.33
20.0	{ 19.2	1.30			
	{ 18.9	1.28			
	{ 20.3	1.22			
	{ 18.7	1.31			

It is clear that there is no consistent relation between temperatures and mean hourly growth rates and that deviations related to unknown influences (including internal variations of the seedlings and errors of observation) must have been sufficiently great to mask any temperature influence that might be evident if all the non-temperature influences had been ideally constant for the series. For the interpretation of the standard-solution controls throughout the entire study it is safe to suppose that such slight temperature fluctuations as may have occurred may be considered as negligible, in relation to the inevitable innate variability of the standard seedlings and possible errors of technique. The average hourly rate of elongation of these roots in standard nutrient solution, for the regular treatment period is 1.27 mm., which is equivalent to an average enlargement of 25 mm. in a period of 20 hrs. The greatest deviation from this average rate was 12.6 per cent. (1.11 mm. in the above tabulation).

CONTROLS WITH DISTILLED WATER.—Very little root elongation occurred in the distilled-water controls during the treatment period and these roots failed to elongate further when returned to standard solution for the recovery period. After about four days they began to produce laterals,

however, showing that the distilled-water treatment had not killed the entire root. The relations between distilled-water cultures and nutrient-solution cultures for the treatment period and the succeeding 10-hr. interval are shown below.

	Elongation in standard nutrient solution	Elongation in distilled water	
		Actual	Percentage index
Treatment period, about 20 hr.	mm. 25.7	mm. 4.3	16.7
10-hr. recovery period	10.0	0.0	0.0

It will be remembered that the standard seedlings (with roots 30 mm. long) had shown a mean rate of elongation of about 1 mm. per hour in preliminary solution culture and that this rate had been nearly the same for the previous 15 mm. of elongation. The control units in standard nutrient solution continued to show this maintained rate of root growth till the end of the recovery period but the control units in distilled water are seen to have been very markedly retarded in the treatment period. Distilled water consequently acted as a highly toxic medium, which is in accord with the statements of many writers who have experimented with distilled water as a medium for plant cultures.

A special series of measurements was made to secure information concerning the manner in which distilled-water retardation first became manifest after the transfer of a seedling from the standard solution. The root of a standard seedling that had been growing in a tube of standard nutrient solution for 2 hours in the experiment room, was measured several times in the next 15 minutes, by means of a horizontal microscope used like a cathetometer. The mean hourly rate of elongation was 1.46 mm., with fluctuation between 1.43 mm. and 1.51 mm. After the mean rate of growth in nutrient solution had been thus ascertained the seedling was quickly rinsed in distilled water and mounted in a tube of this medium, and the microscopic readings were continued for 2 hr. Only half a minute was required for the transfer. The distilled water had the same temperature as the nutrient solution. Its hydrogen-ion concentration was markedly lower than that of the nutrient solution; the pH value of the water was about 5.6, while that of the solution was about 4.4. Promptly after the change of medium the root began to show growth retardation, which gradually increased throughout the period, as is shown by the graph of figure 3.

It is clear from this special series of measurements that injury by distilled water progressed for several hours. In the distilled-water con-

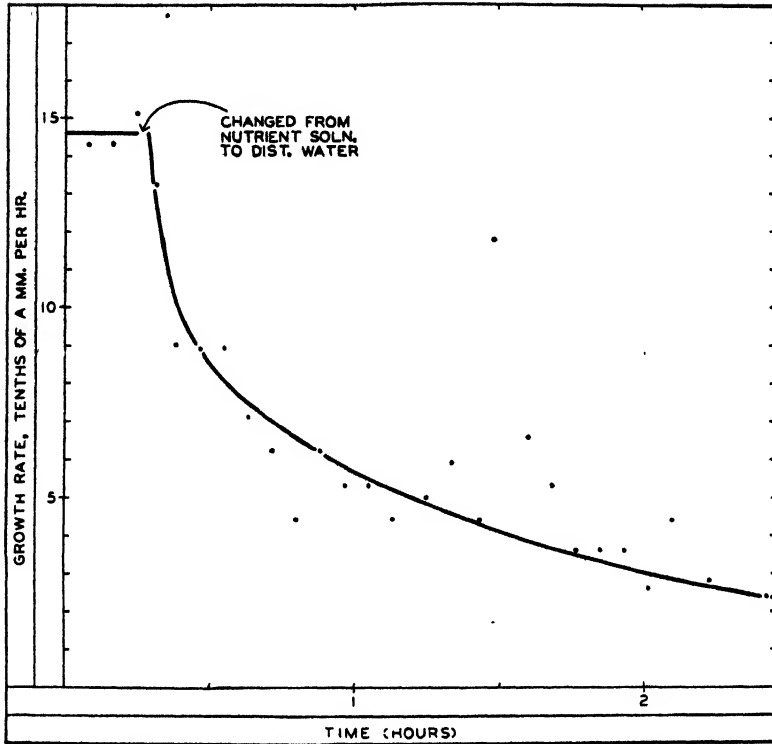


FIG. 3. Graph showing gradual slowing down of primary-root elongation after transfer of a standard seedling from standard nutrient solution to distilled water.

trols of the regular experimentation elongation of the primary root had ceased before the end of the treatment period, the mean length of which was 21.2 hr. The individual roots of the distilled-water control units showed much greater variability than was shown by those of the nutrient-solution units. This may have been partly due to the fact that these measurements were relatively much less precise, because of the small amount of elongation measured, but the same was true of the averages for the several units, and errors of measurement can scarcely be accountable for that, since there were 15 seedlings in every unit. In some instances the treatment-period average for a distilled-water unit was as much as 40 per cent. larger or smaller than the mean of all such averages. Since no average for any nutrient-solution unit deviated from the mean of all such units by more than 8.3 per cent., as has been said, it is clear that the distilled water used acted on these seedlings not only to retard root elongation to a very great degree but also to make it appear that their innate variability was much greater than was apparent from the nutrient-solution tests. It

is generally true that the innate variability of a stock of organisms is more pronounced for somewhat unhealthy or injured organisms than for more healthy ones.

Many writers on toxicity and the chemical stimulation of plants have employed distilled water as the only control medium in their experiments. As has been emphasized by TRUE (31) and SHIVE (24), however, such a control is very unsatisfactory both logically and practically. Distilled waters are apt to differ as to their impurities, and cultures in this medium may therefore be impossible of repetition. Even if laboriously purified by redistillation, etc., distilled water is easily contaminated by contact with the air or in other ways, and of course it cannot remain pure after organisms have been placed in it. Furthermore, for plants with roots in water very low concentrations of toxic solutes generally have much more marked effects in the absence of the essential nutrient salts than when those salts are present in proportions and concentrations suitable to maintain health. Finally, seedlings such as those employed in this study can live but a few weeks with their roots in distilled water. No matter how nearly pure the water may be, the plant must be injured from the start, partly through loss of considerable amounts of essential ions and partly because the necessary absorption of essential mineral nutrients is of course prevented.

The problem of distilled-water toxicity is interesting in itself and it has received much attention (see, for example: LIVINGSTON, 16; SCARTH, 23; MEVIUS, 22), but the results of an investigation of any particular lot of water can refer only to that lot, because of the great difficulty and uncertainty of securing different lots that are alike as to impurities. The importance of this problem practically vanishes, however, when a good nutrient solution is employed as reference basis in studies on nutrition, toxicity and the like. Many workers have noted that very low concentrations of impurities such as may occur in fairly good distilled water exert no observable influences on most organisms when the essential nutrient substances are present in suitably balanced concentrations. With these considerations in mind it was decided to employ modified nutrient solution for the main part of this study. Only one series of tests with solutions that did not contain the three nutrient salts is reported.

Results

GENERAL STATEMENTS

The main results of this study will be presented in the five following subsections. As has been said, they are regularly in the form of percentage indices of primary-root elongation. When the index for a given experiment is 100 it means that the average elongation in that test was the

same as in the corresponding control unit; indices above 100 indicate stimulation (greater average elongation than was given by the corresponding control unit) and indices below 100 show retardation (less elongation than was given by the corresponding control unit). A number of the experiment solutions were tested twice, in different experiments, and the index values given in such instances illustrate the general degree of experimental deviation that should be allowed for in each series. Because the different concentrations of the added compound in any series of experiment solutions form an ascending series of concentrations with relatively small intervals or steps, the weight or significance of the index value for any solution is to be estimated mainly with reference to the values for other solutions above and below it in its series. In spite of considerable deviations, the percentage indices for each series are remarkably consistent in their indications concerning the various degrees to which the rate of primary-root elongation was affected by different concentrations or physiological intensities of the same compound.

The main numerical data are presented in tables I, III, IV, V, VI and VII, and also graphically in figures 4, 6, 7, 8 and 9. In each of these tables the tested concentrations of the added compound are shown in the first column, as millionths normal, the percentage indices for the treatment period are in the second column and those for the 10-hr. recovery interval are in the third. In each figure the two series of values are plotted as ordinates, with abscissas representing the corresponding concentrations. Points for the treatment series are shown as dots and those for the recovery period are circles. Of the two graphs in each of these figures, the full line represents the treatment indices and the broken line represents the recovery indices.

Besides the regular series of experiment solutions for each of the five compounds studied, there were two extra series dealing with acetic acid in special ways. The results of these will appear below.

RESULTS WITH ACETIC ACID

ACETIC ACID IN STANDARD NUTRIENT SOLUTION.—There were 35 different experiment solutions containing acetic acid in addition to the regular nutrient salts, and the partial concentrations due to the acid ranged from 35 to 6,652 millionths normal, as shown in table I. In the same table are presented the percentage indices of primary-root elongation for the treatment period and for the recovery period. The percentage index of growth retardation is naturally always 100 *minus* the elongation index; an elongation of 90 per cent. means a retardation of 10 per cent. Of course the percentage values show much unaccountable fluctuation as one reads either

TABLE I
DATA FOR ACETIC ACID IN NUTRIENT SOLUTION

CONCENTRATION OF ACETIC ACID IN NUTRIENT SOLUTION	RELATIVE AVERAGE ELONGATION EXPRESSED AS PERCENTAGE OF CORRESPONDING CONTROL AVERAGE	
	FOR TREATMENT PERIOD (NUTRIENT SOLUTION WITH ACID)	FOR RECOVERY PERIOD (NUTRIENT SOLUTION WITHOUT ACID)
<i>millionths normal</i>	<i>per cent.</i>	<i>per cent.</i>
35	88.3	102.0
60	97.5	108.0
71	{ 94.8	92.0
	{ 92.6	103.0
106	{ 90.6	98.0
	{ 85.5	95.5
142	{ 89.5	103.0
	{ 87.2	107.0
176	{ 86.2	90.0
	{ 84.0	108.0
212	83.2	94.6
242	90.9	107.0
282	89.5	83.0
302	89.8	100.0
363	98.0	126.0
454	99.4	81.0
544	97.0	103.0
	{ 95.3	99.0
605	{ 100.3	90.0
	{ 106.3	112.2
680	92.5	114.0
726	83.5	93.5
756	{ 91.5	107.0
	{ 94.0	95.1
	{ 95.4	116.5
847	{ 98.5	124.0
907	89.5	112.0
1,062	{ 79.7	131.5
	{ 70.6	113.0
1,129	68.3	112.8
1,210	38.5	64.7
1,512	{ 28.8	45.3
	{ 27.5	34.5
1,663	{ 24.3	24.7
	{ 20.4	15.4
1,814	19.7	3.2
2,117	12.8	0
2,419	12.2	0
3,024	{ 10.7	11.8
	{ 7.5	0
3,629	7.2	0.8
3,928	7.7	3.5
4,838	4.8	0
5,543	1.1	6.3
6,048	{ 3.4	0
	{ 6.2	0
6,350	{ 8.0	0
	{ 10.6	1.5
6,652	0	4.8

column in the table. The broad-line graphs of figure 4 approximately represent the relations in question and the dots indicate the actual data.

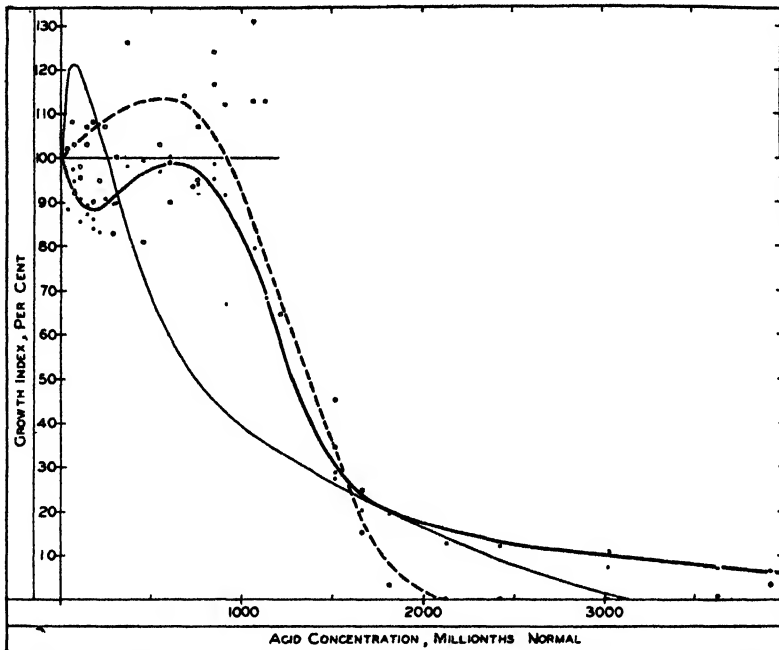


FIG. 4. Graphs showing influence of *acetic acid* on primary-root elongation. The two broad lines represent nutrient solutions to which the acid had been added in various concentrations, dots and the continuous line being for the treatment period while circles and the broken line are for the recovery period; data are from table I. The narrow line represents simple solutions of acetic acid in *distilled water*, for the treatment period; data are from table II.

For the Treatment Period, during which the roots were subject to the direct action of the acid in the medium, the continuous broad-line graph indicates that the experiment solutions with concentrations of acid below about 500 millionths normal gave less root elongation than was given by the control nutrient solution without addition of acid. For this concentration range ordinates are less than 100 and the greatest retardation is indicated for an ordinate of about 88, with acid concentration of about 175 millionths normal. Elongation percentages are shown as less than 90 for the concentration range from about 110 to about 260 millionths normal. After this minimal region is passed the treatment-period graph bends upward and the concentration range from about 500 to about 750 millionths normal shows percentages little below 100; for these solutions there was little or no retardation of growth, and no stimulation is indicated. The

graph then slopes rapidly downward and becomes nearly rectilinear between ordinates of 90 and 30, corresponding to acid concentrations of about 880 and about 1,520 millionths normal, respectively. For this range growth retardation appears to have increased by 1 per cent. for each acid-concentration increase of about 10 millionths normal. The slope then gradually decreases and the graph again becomes a nearly straight line, but now nearly parallel with the x axis, beyond the point for a concentration of 3,000 millionths normal, at which point the ordinate is about 10.

Turning to the Recovery Graph, the broken line of figure 4, which represents the after effect of the acid treatment for the first 10 hr. after treatment had ceased with the return of the seedlings to standard nutrient solution, a pronounced stimulation of growth is indicated for the acid-concentration range below about 920 millionths normal. This stimulation amounts to 10 per cent. or more for the seedlings treated with acid solutions having concentrations between about 300 and about 750 millionths normal. For treatments with acid solutions more concentrated than about 920 millionths normal the recovery index decreases regularly, as indicated by a nearly straight line, until the treatment concentration of the acid reaches about 1,750 millionths normal and the percentage index attains the low value of 10. For this range of acid concentrations, from about 920 to about 1,750 millionths normal, the slope of the graph indicates an increase in retardation, or a decrease in relative elongation, of 1 per cent. for each increase in concentration of about 8 millionths normal. Beyond the ordinate 10 the graph continues to slope downward with concentrations still higher than 1,750 millionths normal, but at a decreasing rate, and the x axis is apparently reached with a concentration of about 2,120 millionths normal. This means that previous treatment with an experiment solution having an acid concentration of about 2,120 millionths normal, or higher, had so injured the primary roots that they failed to elongate at all in the 10-hr. recovery period. All these general indications are of course only approximations, but the actual data are shown by the circles of figure 4.

It is interesting to observe that the two broad-line graphs of figure 4 intersect at about the point for 1,575 millionths normal acid concentration and an elongation index of about 27, which means that treatment with an experiment solution having this concentration of acetic acid produced a direct effect amounting to a growth retardation of 73 per cent. and that its after effect, as here measured, was numerically about the same. However, the two graphs are close together throughout the range of concentrations from about 1,000 to about 1,500 millionths normal and it is probably safe to consider the direct toxicity and the after effect as about alike for this concentration range.

It is obvious that the exact shapes of these two broad-line graphs of figure 4 may be quantitatively more or less in error, especially in the regions representing the double reversal and the occurrence of after-effect stimulation. The evidence for these features should be regarded as tentative until more extensive experimentation may be carried out on the growth behavior of this kind of roots in comparatively low concentrations of acetic acid in nutrient solution. At any rate, a reliable picture of what was actually observed in the present brief study is given by these graphs and their dots and circles.

ACETIC ACID IN DISTILLED WATER.—Nineteen different concentrations of acetic acid without the three nutrient salts were tested with control units in distilled water. These concentrations ranged from 19 to 3,629 millionths normal, as shown in the first column of table II. The numerical results of this series of tests are shown for the treatment period in the second column of the same table, and by the narrow-line graph of figure 4. To avoid con-

TABLE II
DATA FOR ACETIC ACID IN DISTILLED WATER, FOR TREATMENT PERIOD

CONCENTRATION OF ACID	RELATIVE AVERAGE ELONGATION (PERCENT- AGE OF CORRESPOND- ING CONTROL AVERAGE FOR DISTILLED WATER)	CONCENTRATION OF ACID	RELATIVE AVERAGE ELONGATION (PERCENT- AGE OF CORRESPOND- ING CONTROL AVERAGE FOR DISTILLED WATER)
<i>millionths normal</i>	<i>per cent.</i>	<i>millionths normal</i>	<i>per cent.</i>
19	133	907	40
38	{ 121 82	1,210	{ 32 6
76	{ 121 98	1,512	24
151	115	1,814	21
227	102	2,117	15
302	90	2,419	{ 15 6
454	{ 72 51	2,722	{ 12 10
605	{ 60 32	3,024	0
756	57	3,175	0
		3,629	0

fusion the actual points of observation for this graph are not shown. That there were large deviations for the least concentrated solutions is clear from the table.

On the narrow-line graph of figure 4 a pronounced after-effect stimulation is indicated for a range of acid concentrations below about 250 millionths normal and no elongation occurred with solutions more concentrated than 3,100 millionths normal. Beyond the stimulation range acetic acid appears to have been more toxic when used alone in the treatment period than when accompanied by the three nutrient salts. This supports the generalization that the poisonous action of a solute may be greatly retarded or prevented through concomitant action of nutrient salts or other substances in the same solution.

As has been mentioned, the control seedlings that had been in distilled water alone during the treatment period failed to show any root elongation in the recovery period, having been seriously injured by the treatment. Those that had been in simple solutions of acetic acid during the treatment period also failed to show elongation of the primary roots in the 10-hr. recovery period, as would be expected. In the next four days, however, the seedlings from distilled water and those from acetic-acid solutions with concentrations below about 1,512 millionths normal all developed secondary roots, showing that the entire root had not been killed either by the distilled-water treatment or by treatment with the less concentrated simple solutions of this acid.

ACETIC ACID AT CONTINUOUSLY INCREASING CONCENTRATION IN FLOWING NUTRIENT SOLUTION.—In two like experiments of a preliminary nature a seedling root was measured with a horizontal microscope every 15 minutes for 9.25 hr., while a continuously changing experiment solution containing acetic acid and the nutrient salts flowed around and past it at a rate of about 36 ml. per hour. Only one standard seedling was used in each experiment, mounted in the usual manner but in a 50-ml. test tube. The paraffined cork bearing the seedling had two extra perforations, for inlet tube and outlet tube, and the waste solution dripped into a graduated cylinder by means of which the rate of flow was ascertained from time to time. The inlet extended nearly to the bottom of the test tube and the outlet reached only slightly below the upper surface of the cork.

Standard nutrient solution and the same solution to which acetic acid had been added so as to have an acid concentration of 3,024 millionths normal were brought together at the requisite calculated rates from two 50-ml. burettes. The resulting mixture then passed slowly through two glass mixing chambers before being discharged into the culture tube. A very thorough mixing of the two solutions was thus secured and also a very

gradual change in the acid concentration of the slow stream through the culture tube. The rate of flow through the culture tube was kept nearly constant but the flow of acid solution into the mixer was regulated by hand, so that the acid concentration of the mixed solution increased gradually from 0 to about 3,024 millionths normal. These two experiments were performed in the experiment room but the temperature was higher than usual (about 23°) because of a continuously glowing electric lamp and the continuous presence of the operator.

In the beginning the apparatus was filled with standard nutrient solution and several microscopic measurements were made to ascertain the rate at which elongation was taking place in that solution. The average from these preliminary measurements was taken as the basis for computing the percentage increments for succeeding 15-minute intervals after the flowing solution began to change. At the end of the experiment the root was surrounded by a solution containing the nutrient salts just as at the start but with the addition of acetic acid at a concentration closely approaching 3,024 millionths normal.

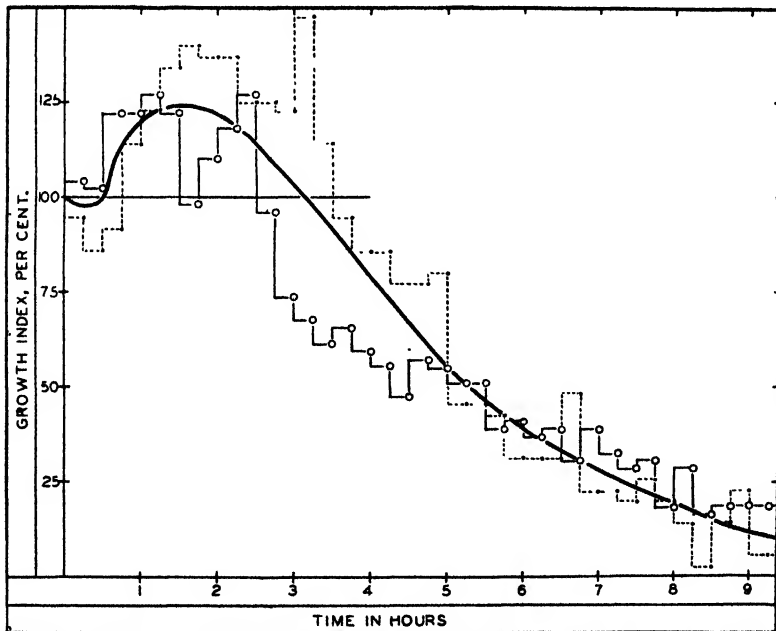


FIG. 5. Graphs showing the march of primary-root elongation in a continuously flowing solution with gradually increasing concentration of *acetic acid* but with the concentrations of the three nutrient salts maintained as in standard nutrient solution. The step graphs represent separate experiments and the curve represents a combination of the two.

The numerical results of these two special experiments are shown graphically in figure 5, where abscissas represent time and ordinates are the elongation percentages. Each percentage value is plotted as at the end of its 15-minute interval, the points for one series being dots and for the other circles. The curve shows the general trend indicated by these two sets of points.

As is readily seen, these two experiments were not in agreement as to details, especially for the first four hours, but they agree in indicating marked stimulation at first (with very low acid concentrations) and increasing retardation afterwards (as the acid concentration in the flowing solution increased). The generalized curve resembles the graphs of figure 4 in some respects but differs from them in others. The four graphs cannot be compared in detail, of course, but their combined evidence shows at least the possibility of marked stimulation of these roots by acetic acid at low concentrations. Whether or not such stimulation was actually shown seems to have depended on the technique employed in experimentation. Many hypothetical explanations of the differences between these four graphs might be elaborated but they need not be presented at this time.

RESULTS WITH PROPIONIC ACID IN NUTRIENT SOLUTION

Twenty-eight different experiment solutions were tested with propionic acid in addition to the regular nutrient salts. The acid concentrations ranged from 30 to 12,090 millionths normal, as shown in the first column of table III. In the second and third columns are presented the percentage indices of root elongation for the treatment period and for the recovery period. This table is arranged like table I and the two graphs of figure 6 approximately represent the numerical values as in the case of the broad-line graphs of figure 4.

For the Treatment Period, the continuous-line graph of figure 6 slopes rapidly downward from the start, without clear indication of stimulation by the solutions of very low acid concentration. It is nearly rectilinear for the concentration range from about 250 to about 750 millionths normal, its slope in this region representing a decrease in the elongation index from about 85 to 30 and a growth retardation of 1 per cent. for each increase in acid concentration of about 6 millionths normal. Beyond the point for an acid concentration of about 750 millionths normal the slope decreases and the graph again becomes nearly rectilinear, and not far from horizontal, for the broad concentration range from about 1,500 to about 12,090 millionths normal. For this whole region the ordinate decreases from about 11 to 0. The acid concentration for a growth index of 90 per cent., (that is, for a retardation of 10 per cent.) is shown as about 190 millionths

TABLE III
DATA FOR PROPIONIC ACID IN NUTRIENT SOLUTION

CONCENTRATION OF PROPIONIC ACID IN NUTRIENT SOLUTION	RELATIVE AVERAGE ELONGATION EXPRESSED AS PERCENTAGE OF CORRESPONDING CONTROL AVERAGE	
	FOR TREATMENT PERIOD (NUTRIENT SOLUTION WITH ACID)	FOR RECOVERY PERIOD (NUTRIENT SOLUTION WITHOUT ACID)
<i>millionths normal</i>	<i>per cent.</i>	<i>per cent.</i>
30	103.0	114.0
40	92.0	100.0
81	113.0	94.0
101	{ 106.0	128.0
	{ 95.0	104.0
121	117.0	113.0
161	83.5	118.0
201	{ 102.0	101.0
	{ 91.3	105.3
302	{ 82.1	68.7
	{ 78.0	62.1
322	72.7	42.3
403	80.7	61.0
504	64.0	35.0
569	45.0	22.9
604	46.0	41.5
705	37.0	22.4
806	28.6	4.5
907	18.6	6.0
1,007	17.6	17.7
1,209	14.5	9.6
1,417	14.2	4.7
1,612	12.2	5.8
1,814	9.8	6.8
2,015	{ 11.7	0.4
	{ 9.2	0
4,030	8.9	2.9
6,045	8.5	4.4
7,254	5.0	1.4
8,060	3.9	5.8
9,672	3.4	0
12,090	0	0

normal, and the concentration for an index of 10 per cent. (a retardation of 90 per cent.) is about 2,200 millionths normal.

This treatment-period graph for propionic acid in nutrient solution differs from the corresponding graph for acetic acid in that it does not show the reversals that characterize the other graph near the start and it does not approach the base line so rapidly in its final, nearly horizontal portion. The graph for propionic acid is much like that portion of the acetic-acid graph which lies beyond the ordinate for a concentration about 600 millionths normal. Neglecting the reversals of the acetic-acid graph, we might say that acetic acid had very little effect in concentrations below

about 600 millionths normal, while propionic acid had a very marked effect in the lowest concentrations tested. Altogether, it appears that propionic acid was considerably more toxic than acetic acid.

For the Ten-Hour Recovery Period, represented by the broken line of figure 6, a pronounced stimulation is indicated for propionic-acid concentrations below about 200 millionths normal and that concentration appears to have had no effect. Beyond this region of stimulation the graph descends very rapidly at first and then more and more slowly. The ordinate for an elongation index of 10 corresponds to about 1,300 on the scale of abscissas and the complete graph meets the base line at the point representing an acid concentration of about 9,500 millionths normal.

This graph resembles the corresponding one for acetic acid but the stimulation range for propionic acid is apparently much narrower. This supports the supposition that propionic acid was the more active physiologically. On the other hand, a much higher concentration of propionic acid was required to prevent any root elongation in the 10-hr. recovery period. This may suggest that the roots recovered from non-lethal poisoning by propionic acid more readily than they did from a like degree of injury produced by acetic acid.

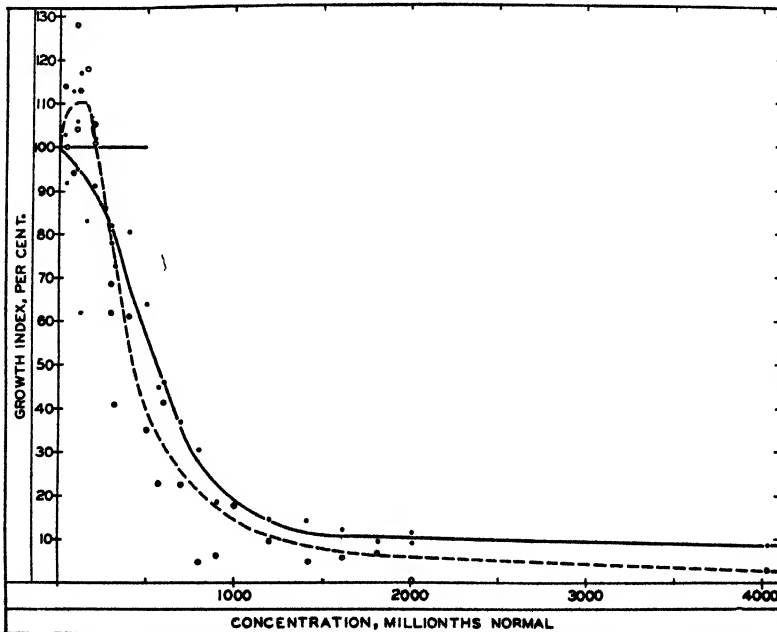


Fig. 6. Graphs showing influence of *propionic acid* on primary-root elongation. Dots and continuous line represent the treatment period while circles and broken line represent the recovery period; data are from table III.

The two graphs for propionic acid intersect at the point for an acid concentration of about 270 millionths normal and a percentage index of about 85. Beyond this point they lie close together, the after-effect graph being below the other.

The dots of figure 6 suggest some stimulation in the treatment period but the curve does not take this suggestion into account. There seems to be no doubt of the occurrence of after-effect stimulation, although the initial convex portion of the broken-line graph, representing this stimulation, may be somewhat too low or too high.

RESULTS WITH NORMAL BUTYRIC ACID IN NUTRIENT SOLUTION

The experiment solutions with normal butyric acid in nutrient solution were 26 in number, ranging in acid concentration from 47 to 4,714 millionths normal. The data for this series of experiments are presented in table IV and in figure 7, arranged as are the data in the corresponding tables and figures for acetic acid and propionic acid.

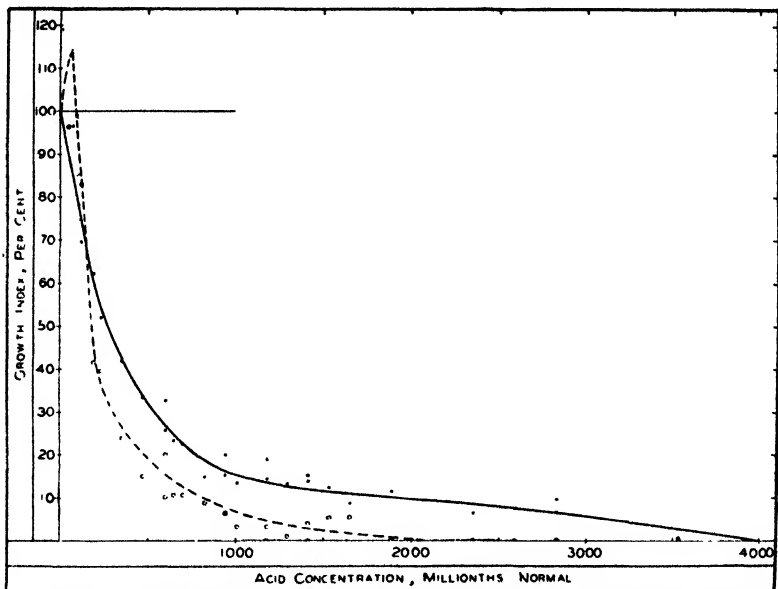


FIG. 7. Graphs showing influence of *normal butyric acid* on primary-root elongation. Dots and continuous line represent treatment period while circles and broken line represent recovery period; data are from table IV.

For the Treatment Period, the continuous-line graph of figure 7 is essentially like that for propionic acid but it descends somewhat more rapidly. There is no suggestion of any butyric-acid stimulation in the treatment period. The graph meets the base line at the point for an acid concentra-

TABLE IV
DATA FOR NORMAL BUTYRIC ACID IN NUTRIENT SOLUTION

CONCENTRATION OF N. BUTYRIC ACID IN NUTRIENT SOLUTION	RELATIVE AVERAGE ELONGATION EXPRESSED AS PERCENTAGE OF CORRESPONDING CONTROL AVERAGE	
	FOR TREATMENT PERIOD (NUTRIENT SOLUTION WITH ACID)	FOR RECOVERY PERIOD (NUTRIENT SOLUTION WITHOUT ACID)
<i>millionths normal</i>	<i>per cent.</i>	<i>per cent.</i>
47	96.0	96.2
71	96.3	114.0
94	95.1	98.8
106	78.0	85.0
118	69.4	83.0
189	62.2	41.8
236	52.0	38.6
353	41.9	23.9
471	33.3	15.1
599	{ 32.8	20.0
	{ 25.8	10.1
648	23.6	10.7
707	22.6	10.8
825	15.1	8.6
943	{ 20.1	6.2
	{ 15.5	6.4
1,061	13.6	3.2
1,178	{ 19.3	0
	{ 14.7	3.1
1,296	13.2	1.1
1,414	{ 15.2	4.1
	{ 14.0	0
1,532	12.4	5.4
1,650	8.9	5.6
1,885	11.5	0
2,357	6.7	0
2,593	7.8	0
2,828	{ 9.9	0
	{ 6.7	0
3,535	0.7	0.6
4,714	0.8	3.1

tion of about 4,000 millionths normal. Butyric acid consequently seems to have been more toxic than propionic acid. The concentration for an elongation index of 90 is shown as about 30 millionths normal and that for an index of 10 appears as about 2,000 millionths normal.

For the 10-Hour Recovery Period, the graph for butyric acid would be nearly like the corresponding one for propionic acid if the latter were moved a little to the left. The broken-line curve is drawn to indicate stimulation for a very narrow range of the lowest concentrations (below about 85 millionths normal) but this is really no more than a suggestion, for the evidence therefor is just the single percentage value for the concentration 71 millionths normal.

This butyric-acid graph reaches the base line at the point for a concentration of about 2,080 millionths normal. Both of these butyric-acid graphs, when compared with the corresponding ones for propionic acid, indicate that butyric acid was somewhat more active physiologically than was the other acid.

The two graphs of figure 7 intersect at the point representing an acid concentration of about 150 millionths normal and a percentage index of elongation of about 69.

RESULTS WITH SULPHURIC ACID IN NUTRIENT SOLUTION

Eighteen different concentrations of sulphuric acid were tested in standard nutrient solution, ranging from 43 to 2,583 millionths normal. The results are shown in table V and figure 8.

TABLE V
DATA FOR SULPHURIC ACID IN NUTRIENT SOLUTION

CONCENTRATION OF SULPHURIC ACID IN NUTRIENT SOLUTION	RELATIVE AVERAGE ELONGATION EXPRESSED AS PERCENTAGE OF CORRESPONDING CONTROL AVERAGE	
	FOR TREATMENT PERIOD (NUTRIENT SOLUTION WITH ACID)	FOR RECOVERY PERIOD (NUTRIENT SOLUTION WITHOUT ACID)
<i>millionths normal</i>	<i>per cent.</i>	<i>per cent.</i>
43	99.0	115.0
65	98.3	113.0
108	94.5	90.0
172	90.9	99.0
215	90.0	83.8
323	84.0	64.8
430	74.5	57.6
495	64.7	42.8
538	59.1	49.0
646	29.7	29.6
753	18.1	5.1
861	11.3	1.5
1,076	7.5	5.1
1,292	7.6	2.0
1,477	2.9	5.1
1,722	2.5	0
2,152	1.1	0
2,583	0	5.1

For the Treatment Period, the sulphuric-acid graph (continuous-line of figure 8) follows the corresponding graph for propionic acid very closely in the region of acid concentrations from 0 to about 500 millionths normal.

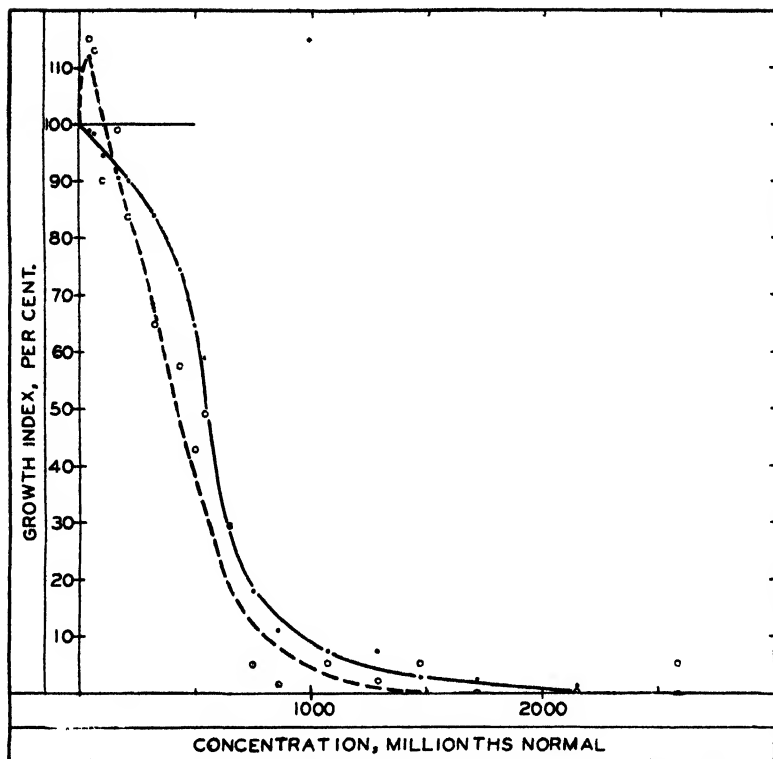


FIG. 8. Graphs showing influence of sulphuric acid on primary-root elongation. Dots and continuous line represent treatment period while circles and broken line represent recovery period; data are from table V.

It then descends more rapidly than the one for propionic acid and reaches the base line about the point for an acid concentration of 2,120 millionths normal.

For concentrations below about 500 millionths normal the toxicity of this mineral acid appears to have been about like that of propionic acid and for higher concentrations sulphuric acid was apparently considerably more toxic than either of the organic acids tested. For an elongation percentage of 90 the concentration of sulphuric acid is shown as about 210 millionths normal and for a percentage of 10 (a retardation of 90 per cent.) it appears to have been about 950 millionths normal. There is no suggestion of any stimulation by sulphuric acid in the treatment period.

For the Ten-Hour Recovery Period, the graph for sulphuric acid (the broken line of figure 8) is drawn to indicate stimulation for a narrow range of low acid concentrations, very much as are the corresponding graphs for propionic acid and butyric acid. But actual evidence for stimulation is

not more significant here than in the corresponding case of normal butyric acid (observe the circles at the extreme left). We should say that after-effect stimulation by sulphuric acid is no more than suggested by the data.

Beyond the region of suggested stimulation the graph for sulphuric acid closely follows the corresponding one for propionic acid as far as the point representing a concentration of about 500 millionths normal and an elongation index of about 39. From that point onward it descends more rapidly than the corresponding propionic-acid graph and meets the base line at a point for a concentration of about 1,430 millionths normal. In this respect it is similar to the recovery graphs for both acetic acid and normal butyric acid.

The two graphs of figure 8 intersect at the point representing an acid concentration of about 150 millionths normal and a percentage index of elongation of about 93.

RESULTS WITH POTASSIUM ACETATE IN NUTRIENT SOLUTION

Nineteen different concentrations of potassium acetate in standard nutrient solution were tested, ranging from 228 to 15,230 millionths normal. The results of these tests are shown in table VI and figure 9.

For the Treatment Period, the acetate graph (the continuous line of figure 9) shows no stimulation and the toxicity of this compound appears

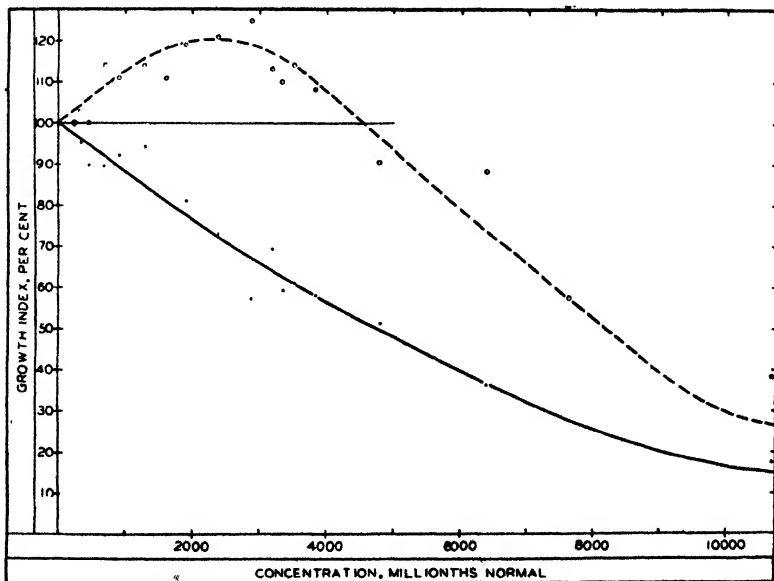


FIG. 9. Graphs showing influence of *potassium acetate* on primary-root elongation. Dots and continuous line represent treatment period while circles and broken line represent recovery period; data are from table VI.

to have been proportional to its concentration from 0 to about 7,500 millionths normal, with some decrease in slope indicated for still higher concentrations. For the concentration range just mentioned the elongation index appears to decrease 1 per cent. for an increase in concentration of about 105 millionths normal. As might be expected, the toxicity of the acetate is clearly very different in its concentration relations from the toxicity of any of the acids tested. The concentration giving a growth index of 90 was about 840 millionths normal. The highest concentration in the series (15,230 millionths normal) was not high enough to inhibit root elongation in the treatment period, for it gave an average percentage index of 10.6.

TABLE VI
DATA FOR POTASSIUM ACETATE IN NUTRIENT SOLUTION

CONCENTRATION OF ACETATE IN NUTRIENT SOLUTION	RELATIVE AVERAGE ELONGATION EXPRESSED AS PERCENTAGE OF CORRESPONDING CONTROL AVERAGE	
	FOR TREATMENT PERIOD (NUTRIENT SOLUTION WITH ACETATE)	FOR RECOVERY PERIOD (NUTRIENT SOLUTION WITHOUT ACETATE)
<i>millionths normal</i>	<i>per cent.</i>	<i>per cent.</i>
228	99.7	100.0
318	95.4	103.0
457	89.8	100.0
685	89.5	114.0
914	92.0	111.0
1,272	94.1	114.0
1,599	78.5	111.0
1,908	81.0	119.0
2,385	73.0	121.0
2,862	57.3	125.0
3,180	69.3	113.0
3,339	59.4	110.0
3,498	61.0	114.0
3,816	58.0	108.0
4,770	51.2	90.7
6,360	36.3	88.0
7,615	26.7	57.5
10,660	17.8	38.2
15,230	10.6	20.5

For the Ten-Hour Recovery Period, the acetate graph (the broken line of fig. 9) shows pronounced stimulation, but it differs markedly from the others in that the range of concentrations that gave stimulation in this instance is very broad, extending from about 0 to about 4,500 millionths normal. It is specially notable that two solutions as different as the stand-

ard nutrient solution and the one containing the acetate at the relatively high concentration just mentioned should have been alike physiologically, as far as the average growth index indicates; both of them are shown as giving standard growth, with index of 100. Between these two limits of the stimulation range the graph is approximately symmetrical and the range from about 1,900 to about 2,900 millionths normal is shown as giving maximal stimulations of about 20 per cent. For acetate concentrations above the stimulation range the recovery graph descends as a nearly straight line with a slope slightly more rapid than that of the treatment graph. The highest acetate concentration tested gave an average elongation index of 20.5 for the recovery period; that is, a retardation of only about 80 per cent.

In spite of some considerable deviations the dots and circles of figure 9 show that the shapes of the two graphs are not far from correct, for the data at hand, and there seems to be no room for doubt that, while absence of stimulation characterized the treatment period, stimulation was clear and definite for the recovery period. Of course the critical concentration values mentioned above are intended to be only fairly approximate.

ASSEMBLED GRAPHS FOR ALL SERIES BASED ON STANDARD NUTRIENT SOLUTION

The graphs for all five series of modified nutrient solutions are collected in figures 10 and 11 for ready comparison. These are simply reproduc-

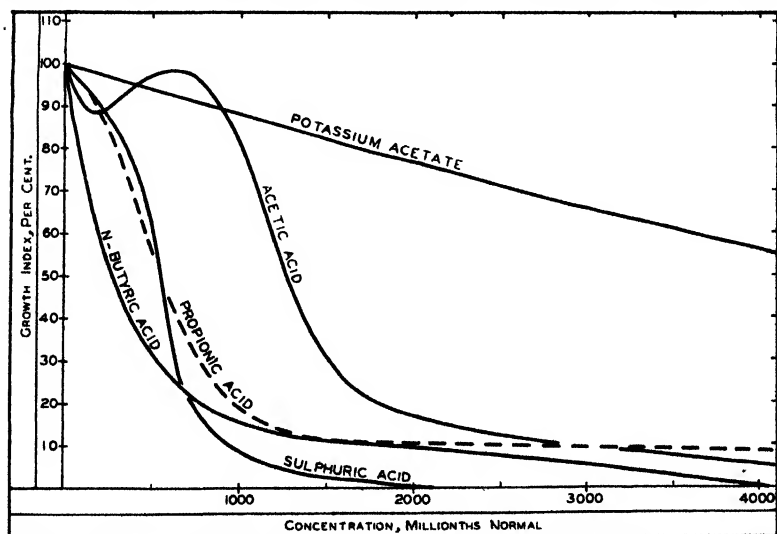


FIG. 10. Graphs showing influence of the five compounds studied, for the treatment period. Here are brought together the continuous-line graphs of figures 4, 6, 7, 8 and 9.

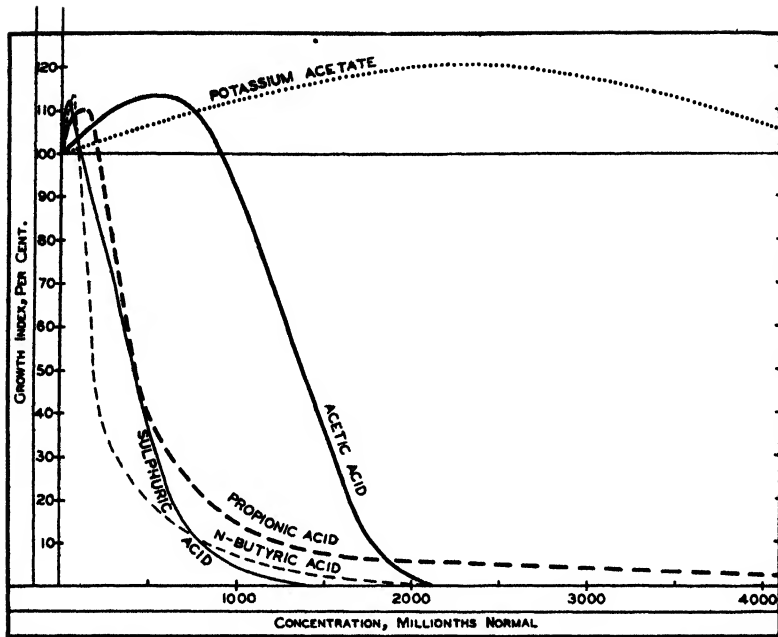


FIG. 11. Graphs showing after effects of the five compounds studied, as shown in 10-hr. recovery period. Here are brought together the broken-line graphs of figures 4, 6, 7, 8 and 9.

tions of the graphs of figures 4, 6, 7, 8 and 9 and they need no special explanations. Figure 10 represents direct effect, in treatment period, and figure 11 represents after effect, in recovery period.

COMPARATIVE TOXICITIES OF THE FIVE COMPOUNDS STUDIED

CONCENTRATIONS SHOWING GROWTH RETARDATION OF 50 AND OF 90 PER CENT. IN THE TREATMENT PERIOD.—In order to secure some simple numerical values to represent the relative toxicities of the five compounds studied we may consider the concentrations corresponding to growth retardations of 50 and 90 per cent. in the treatment period and each toxicity index may be expressed in terms of the reciprocal of the corresponding concentration for acetic acid taken as unity. The results of these computations are shown on page 429. Scales of toxicity more or less different from this would of course result if the comparisons were made with respect to other degrees of growth retardation.

TIME RELATIONS OF TOXICITY AND STIMULATION.—As has been shown, the rate of primary-root elongation decreased gradually following the placing of standard seedlings in distilled water, while those kept in standard nutri-

Compounds added to nutrient solution	Relative toxicities (for treatment period) of modified nutrient solutions giving	
	Growth retardation of 50 per cent.	Growth retardation of 90 per cent.
Acetic acid (CH_3COOH)	1.00	1.00
Propionic acid ($\text{CH}_3\text{CH}_2\text{COOH}$)	2.34	1.50
Normal butyric acid ($\text{CH}_3\text{CH}_2\text{CH}_2\text{COOH}$)	4.59	1.71
Sulphuric acid (H_2SO_4)	2.34	3.16
Potassium acetate (CH_3COOK)	0.27	0.20

ent solution maintained an approximately uniform rate of elongation throughout the whole experiment period, including both the treatment period (about 20 hr.) and the recovery period (about 10 hr.). For the 126 experiment solutions tested, growth increments were measured only for the treatment period and for the recovery period. No information is available as to how the rate of root elongation may have changed in either one of these observation intervals, but it was surely not generally maintained throughout either interval, excepting in the nutrient-solution controls and in the tests that gave the percentage index of about 100. The roots were elongating at the rate of about 1 mm. per hour when they were placed in the experiment solutions and all of the solutions containing the nutrient salts showed a growth retardation for the treatment period. This retardation may have come on gradually, somewhat as is shown for standard seedlings transferred from nutrient solution to distilled water. It is possible, however, that growth rates may have been accelerated in the first part of the treatment period and then sufficiently retarded in the latter part to show retardation for the period taken as a whole. If the regular treatment period had been shorter than it was and of the right length, treatment stimulation might have been shown for the weakest solutions of every series. This is suggested by the pronounced after-effect stimulation shown or suggested for all of the compounds tested and by the marked temporary stimulation shown at the beginning in the experiments with gradually increasing concentrations of acetic acid.

If temporary stimulation may have occurred with some solutions at the beginning of the treatment period, to be obliterated in the records by subsequent retardation before the end of the period, it is equally possible that a first retarding influence may have been followed either by a decrease

in retardation (possibly amounting to stimulation) or by an increase. Such a progressive increase in growth retardation as time went on was shown for roots in the distilled-water controls. KAHLENBERG and TRUE and other experimenters have pointed out that a somewhat toxic solution—or other injurious but non-lethal environmental complex—may, in some instances at least, produce injury only in the early part of the exposure period, the organism eventually recovering or becoming acclimated to conditions that were temporarily injurious at the beginning of the exposure. Thus a root might at length grow well in a solution that at first exerted a pronounced retarding influence.

From these and other considerations it is clear that the time factor needs to be taken into account in the interpretation of the results of such a study as this and it is to be borne in mind that this important factor was among the influential background conditions of the main series of experiments here reported. To test these suggested possibilities would of course require much more extensive and elaborate experimentation than was planned for the present study.

THE POSSIBILITY OF SYNERGISTIC EFFECTS.—It has been shown that the three nutrient salts exerted a pronounced influence on the apparent physiological influence of acetic acid, for the acetic-acid series with these salts gave results for the treatment period very different from those given by the corresponding series without the salts (compare broad-line graph with the narrow-line graph of figure 4). It may be supposed that similar effects of the nutrient salts may be involved in the results secured with the other added compounds. In comparing the toxic and stimulating influences shown in this paper the presence of the nutrient salts in the specified proportions and total salt concentration needs, of course, to be borne in mind. This suggestion involves the possibility of synergistic influences exerted by the many kinds of molecules and ions in these experiment solutions. It will be superficially considered in the section on hydrogen-ion influences.

RELATIONS BETWEEN DIRECT EFFECTS (TREATMENT PERIOD) AND AFTER EFFECTS (RECOVERY PERIOD).—Although the records for the treatment period generally fail to show direct stimulation for any of the series of solutions containing the nutrient salts, the recovery graphs for these series all agree in showing or at least suggesting after-effect stimulation, which is of course confined to the lower concentrations of the added compounds, acting in the treatment period. The concentration limits below which some after-effect stimulation in the recovery period is indicated are approximately as follows:—

Acetic acid	920 millionths normal.
Propionic acid	210 millionths normal.

Normal butyric acid	100 millionths normal (?)
Sulphuric acid	110 millionths normal (?)
Potassium acetate	4,470 millionths normal.

(The interrogation points in parentheses recall the observations already made, that the experimental data are of low significance with respect to after-effect stimulation by normal butyric acid and sulphuric acid. While there is uncertainty as to its degree, after-effect stimulation appears to be clearly shown by the actual data for acetic acid, propionic acid and potassium acetate.)

For solutions having about these critical concentrations of the respective compounds no effect at all is shown for the recovery period but these solutions are all shown to produce growth retardation in the treatment period, as is also true of the less concentrated solutions that gave after-effect stimulation. This means that direct retardation in the treatment period might be followed in the recovery period by either stimulation, absence of effect or retardation, according to the treatment concentration. For all the regular experiment solutions excepting the critical ones approximately specified above, the treated seedlings were apparently altered physiologically to such a degree that in the 10 hr. following cessation of treatment their roots elongated either more or less rapidly than did the roots of the control seedlings, which had been in standard nutrient solution throughout the treatment period. Considering the solutions that are shown as toxic in the recovery period, their after-effect retardation might be either less than, greater than, or equal to the retardation produced directly while they were in contact with the roots. For the four acids tested, the concentrations that show equal growth indices for the two periods are given below, along with their respective growth indices.

	Approximate concentration (millionths normal)	Growth index
Acetic acid	1,575	27
Propionic acid	270	85
Normal butyric acid	150	69
Sulphuric acid	150	93

Finally, we may compare the acid concentrations that are shown as just inhibiting primary-root elongation in the two periods, as shown on page 432. It is seen that this limiting concentration is in every case much lower for the recovery period than for the treatment period. For example, to pre-

	Approximate concentration in experiment solution just preventing root elongation	
	In treatment period	In recovery period
Acetic acid	6,650	2,120
Propionic acid	12,090	9,500
Normal butyric acid	4,060	2,080
Sulphuric acid	2,120	1,430

vent any measurable growth in the treatment period apparently required an acetic-acid concentration of about 6,650 millionths normal, but treatment with this acid at a concentration of only about 2,120 millionths normal is shown as preventing root elongation in the period following the cessation of treatment. Concentrations of this acid between these two limits apparently permitted more or less root elongation in the earlier portion of the treatment period but killed the elongating region of the root by the end of the period, or at least rendered it incapable of any elongation in the next 10 hr. following its return to standard nutrient solution. Similar statements apply to the other acids.

A kind of after-effect stimulation apparently somewhat similar to what was encountered in the present study has been described by HILDEBRANDT and BOYCE (11) for alcohol production by yeast in the fermentation of cane molasses. These authors tested MnSO_4 , CuSO_4 and NaCN and found that each of these salts, when present at a suitable concentration in molasses solution containing growing yeast, apparently affected the yeast so treated in such a manner as to show unusually great alcohol production in an unmodified molasses solution that was subsequently inoculated with the treated yeast. In some of these instances there had been some stimulation in the treatment period (seed-yeast culture, with the added salt) but stimulation was more pronounced in the recovery period (principal fermentation stage). In other instances, notably with MnSO_4 , seed yeast that had been markedly *retarded* by the salt in the seed-culture produced considerably more alcohol after transfer to the principal fermentation (without the salt) than was produced in the control, which had been seeded with untreated yeast. The salt treatment apparently altered the seed yeast so that its offspring in the second stage was much more vigorous than the poisoned seed culture had been and also more vigorous than the offspring of ordinary seed cultures.

Relations between physiological influence and hydrogen-ion concentration

THE GENERAL PROBLEM OF ION AND MOLECULE CONCENTRATIONS

The many modified nutrient solutions tested in this study differed primarily with respect to the added compounds and their concentrations. From the viewpoint of physical chemistry their main effective differences cannot be satisfactorily described without reference to their respective concentrations of ions and of undissociated molecules. A large proportion of the solute material in any of these solutions must have been dissociated, and a little of the solvent water also. According to the dissociation theory we suppose that the standard nutrient solution itself contained the following kinds of molecules and ions, besides those that may have arisen from interaction of the nutrient salts among themselves and with the experiment compounds (if there were any such), and also any small traces of other kinds due to impurities that may have been present in the distilled water or in the salts used:—

Molecules of a $\text{Ca}(\text{NO}_3)_2$ and the ions Ca^{++} and NO_3^- resulting from their dissociation.
Molecules of KH_2PO_4 and the ions K^+ , H_2PO_4^- , HPO_4^- , H^+ , and PO_4^- resulting from their dissociation.

Molecules of MgSO_4 and the ions Mg^{++} and SO_4^- resulting from their dissociation.
Molecules of H_2O and the ions H^+ and OH^- resulting from their dissociation.

In each of the four series of experiment solutions consisting of standard nutrient solution with an added organic compound there must have been present, in addition to these inorganic molecules and ions, one kind of organic molecule (*i.e.*, either CH_3COOH , CH_3COOK , $\text{CH}_3\text{CH}_2\text{COOH}$ or $\text{CH}_3\text{CH}_2\text{CH}_2\text{COOH}$) and also its particular anion (*i.e.*, either CH_3COO^- , $\text{CH}_3\text{CH}_2\text{COO}^-$, or $\text{CH}_3\text{CH}_2\text{CH}_2\text{COO}^-$). In the series of solutions containing potassium acetate the amount of K was increased above the amount in the unmodified nutrient solution. In the series containing sulphuric acid the amount of SO_4 was increased but no kind of ion was intentionally added which was not already present in the solution.

A thorough analysis of the relations between the observed physiological effects and the chemical characteristics of the solutions bringing them about would of course have to take into account the actual concentrations of all the kinds of particles present—both the undissociated molecules and their ions. But that kind of analysis of such complicated chemical systems as those here dealt with is practically impossible at present. On the basis of certain assumptions and by means of published tables of apparent ionization percentages in simple solutions of the substances considered, the

molecular and ionic concentrations of these solutions may be calculated with some degree of probability, but the necessary considerations are complicated by the interrelations between the several compounds in the same solution.

COMPARISONS OF THE FIVE SERIES OF SOLUTIONS WITH RESPECT TO
TOXICITY, pH VALUE AND CONCENTRATION OF ADDED COMPOUND

For such complex aqueous solutions as these the only ion concentrations that now lend themselves to ready estimation by direct measurement are those of hydrogen ions and hydroxyl ions. These are of course interdependent, the product of their concentrations being a constant. Table VII shows, for the treatment period, the relations of growth retardation (the difference between growth index and 100) to concentration of the added compound and to pH value, for each series of solutions. These values were read from the smooth graphs of figures 1 and 10. Opposite each retardation percentage shown in the first column one may read in the other columns the corresponding approximate concentration and pH value for each of the five different series of solutions. It is to be remembered that the growth index of 100 always represents the standard nutrient solution, with a pH value of 4.4, and that all these data refer to the treatment period of the experiments, not to the recovery period. This arrangement shows how, for any degree of growth retardation, the concentration of the added compound and the pH value of the experiment solution varied from series to series. The concentrations shown for any given degree of toxicity are generally different for different compounds, although there is fairly close agreement between propionic acid and sulphuric acid excepting for the greatest retardations or lowest growth indices. The five series of pH values given in table VII are shown graphically in figure 12, where the growth indices are plotted as ordinates and abscissas represent pH values. There is a separate graph for each series of solutions.

We may conveniently consider toxicity as measured, for the given conditions, by the corresponding percentage of growth retardation. Solutions without toxicity show a retardation percentage of 0 and a growth index of 100, and those with the highest degree of toxicity show a retardation percentage of 100 and a growth index of 0. Of course the standard nutrient solution (with zero concentration of added compound and pH value of 4.4) is considered as showing no retardation and is the basis for comparison.

The series for propionic acid and normal butyric acid agree in showing high degrees of toxicity (up to a retardation of 80 per cent.) without any significant alteration in pH value due to the added compound. For these

TABLE VII
COMPARISONS OF ROOT ELONGATION IN THE TREATMENT PERIOD, CONCENTRATION OF ADDED COMPOUND AND PH VALUE, FOR ALL SERIES OF MODIFIED NUTRIENT SOLUTIONS

GROWTH RETARDATION IN TREATMENT PERIOD	APPROXIMATE CONCENTRATIONS OF ADDED COMPOUNDS AND PH VALUES FOR SOLUTIONS GIVING THE DEGREES OF GROWTH RETARDATION SHOWN IN THE FIRST COLUMN										
	ACETIC-ACID SERIES		PROPIONIC-ACID SERIES		BUTYRIC-ACID SERIES		SULPHURIC-ACID SERIES		POTASSIUM- ACETATE SERIES		
	CONCENTRATION	millionths pH normal	CONCENTRATION	millionths pH normal	CONCENTRATION	millionths pH normal	CONCENTRATION	millionths pH normal	CONCENTRATION	millionths pH normal	
Per cent.											
0	0	500-700	4.40	0	4.40	0	4.40	0	4.40	0	4.40
		110									
10		270	4.40	190	4.40	30	4.40	210	4.10	840	5.04
		880									
20		1,020	4.38	300	4.40	85	4.40	350	3.93	1,670	5.25
30		1,110	4.36	385	4.40	140	4.40	450	3.84	2,590	5.34
40		1,185	4.35	465	4.40	200	4.40	510	3.80	3,580	5.39
50		1,285	4.34	550	4.40	280	4.40	550	3.77	4,800	5.56
60		1,370	4.33	650	4.40	380	4.40	590	3.74	6,000	5.65
70		1,520	4.30	780	4.40	530	4.40	640	3.71	7,250	5.70
80		1,800	4.26	965	4.40	780	4.40	730	3.66	8,980	5.72
90		3,000	4.10	2,000	4.17	1,750	4.21	950	3.57	15,300	5.80
100		6,650	3.60	12,090	3.30	4,060	3.74	2,120	3.35		

While this critical concentration differs markedly for these acids the corresponding pH values are relatively not very different and all three are between 0.19 and 0.34 below the pH value for standard nutrient solution. On the other hand, a growth retardation of 50 per cent. corresponds to the following concentrations and pH values:—

	Concentration (millionths normal)	pH
Acetic acid	1,285	4.34
Propionic acid	550	4.40
Normal butyric acid	280	4.40

For this degree of retardation the last two of the acid solutions show the same pH value as the standard nutrient solution, while the first shows a pH value only 0.06 below that of the standard solution. If we consider toxicity as proportional to the reciprocal of the acid concentration corresponding to a growth retardation of 50 per cent. the relative toxicities of these three acids are: acetic acid, 1.0; propionic acid, 2.3; normal butyric acid, 4.6. It is remarkable that the least toxic of the three (acetic acid) is the one showing 4.34 as its critical pH value for a retardation of 50 per cent.

If the growth indices for the first 10 hr. after the treated roots were returned to nutrient solution are compared with the corresponding pH values of the treatment solutions the lack of any general and direct relation between after effect and pH value is very obvious. Those comparisons need not be discussed here but it may be mentioned that the after-effect stimulations indicated for at least three series of solutions would greatly complicate any attempted analysis of the relations between physiological action in the recovery period and hydrogen-ion concentration in the treatment period.

It seems clear that the toxicities of these three fatty acids cannot be estimated by reference to pH value alone. The view that the toxic properties of organic acids are mainly due to undissociated molecules rather than to ions was expressed by J. F. CLARK (3), who experimented with filamentous fungi. He found that acetic acid was about twice as toxic as sulphuric acid, although the former was only 1 per cent. dissociated and the latter was 90 per cent. dissociated in the solutions in question. The results of the present study on root elongation lead to a similar conclusion. MARY E. COLLETT (5), working with *Paramoecium* and *Euplotes*, reached the conclusion that organic-acid molecules were the agents of the toxic action of these acids in her solutions. In reporting experiments on the fungus

Sclerotinia cinerea, DUNN (8) regarded hydrogen-ion concentration as the principal toxicity factor for the common mineral acids but considered the undissociated molecules as of chief importance for the toxicity of the fatty acids. UPPAL (32) reached the same conclusion from a study on the germination of spores of the fungus *Phytophthora*. The results of the present study seem to furnish additional evidence in favor of the conclusions of DUNN and UPPAL.

Summary

This paper reports the main results of an experimental study on the influence exerted by acetic acid, propionic acid, normal butyric acid, sulphuric acid and potassium acetate upon the elongation of primary roots of young seedlings of white lupine (*Lupinus albus* L.). The experimentation was carried on at the Laboratory of Plant Physiology of the Johns Hopkins University in 1929-30.

The seedlings used were all very nearly alike, having been selected from lots that had been grown from selected seeds under specified conditions. Their primary roots were about 30 mm. long and their hypocotyls were about 10 mm. long. At the beginning of each experiment 15 standard seedlings were transferred from preliminary culture in standard nutrient solution to as many separate tubes of one of the experiment solutions, where they remained for a treatment period of about 20 hr. For control units the tubes contained standard nutrient solution. At the close of the treatment period the seedlings were returned to the preliminary-culture jar containing standard nutrient solution, for a 10-hr. recovery period at the end of which after effects of the treatment were observed.

The main series of numerical data are the amounts of primary-root elongation that occurred in the treatment period and in the recovery period. These data are always averages from the 15 seedlings of single experiment units. They are expressed as relative indices of elongation, the corresponding average for the control unit always being taken as 100. Acceleration of growth (stimulation) is thus indicated by index values above 100, growth retardation is shown by values below 100 and index values of 100 indicate that the experiment solution used had no considerable effect on root elongation in the time interval considered. There are two growth indices for each experiment solution, one showing the direct effect of the solution in the treatment period while the other shows any after effect brought out in the 10-hr recovery period.

The standard nutrient solution, which was used generally for controls and as basis for the experiment solutions, was a 3-salt solution containing $\text{Ca}(\text{NO}_3)_2$, KH_2PO_4 and MgSO_4 , in molar concentrations of 0.0050, 0.0069 and 0.0094, respectively. With the specified standard technique the rate

of elongation of roots in this solution (*i.e.*, the average rate for control units) was approximately maintained throughout both time intervals, at a little more than 1 mm. per hour. Any considerable deviations from this rate, in either of the time intervals, must have been due to influences exerted by the experiment solutions in the treatment period.

There were five main series of experiment solutions based on standard nutrient solution. The salt content was alike for all of these but every series differed from each of the remaining four with respect to the nature of an additional compound. The many solutions of each series differed among themselves only with respect to concentration of the additional compound. Thus, for example, the propionic-acid series of experiment solutions were all standard nutrient solution modified to different degrees by the addition of propionic acid to give various concentrations of the acid. The concentrations of the additional compound were so chosen in each series that the whole concentration range was covered, from zero (standard nutrient solution without added compound, in control cultures) to a concentration high enough to prevent root elongation in the treatment period.

Besides the five main series of modified nutrient solutions there were some experiments on simple solutions of acetic acid in distilled water, with distilled-water controls. In those instances the experiment solutions were like those of the main series excepting that the three nutrient salts of the standard nutrient solution were not present.

The numerical results of this study are set forth by means of tables and graphs, which show many interesting relations. For the treatment period the modified nutrient solutions generally gave growth retardation, which was progressively more pronounced as the concentration of the additional compound was higher, until root growth was stopped altogether. Of course the degree of toxicity shown by any of these solutions that did not stop growth varied with the nature of the additional compound as well as with its concentration.

The acetic-acid series of modified nutrient solutions is an exception to this generalization. Although solutions with very low concentrations of this compound gave notable retardation, those with somewhat higher concentrations showed practically no effect and those with still higher concentrations showed retardation. In the second range of concentrations producing retardation the degree of toxicity was greater as the acid concentration was higher and growth was stopped with the highest concentrations tested. Consequently, if abscissas represent concentrations of acetic acid in modified nutrient solution and ordinates are indices of root elongation, the treatment-period graph exhibits two reversals of direction. No such reversals appear in the graphs for the other four series of modified

nutrient solutions; the other acids and the acetate were generally somewhat toxic at low concentrations and progressively more toxic at higher concentrations until the concentration was high enough to prevent growth. There was, however, some suggestion of stimulation by very low propionic-acid concentrations in the treatment period.

The series of simple solutions of acetic acid in distilled water—without the salts of the nutrient solution—was the only series that surely showed stimulation in the treatment period. This is indicated for a narrow range of low acid concentrations. Solutions of somewhat higher concentrations produced little or no effect while those of still higher concentrations retarded growth, as in the higher concentration ranges of the other series.

For the first 10 hr. following the treatment period, after the plantlets had been rinsed and returned to standard nutrient solution, the roots that had been treated with acetic acid, propionic acid or potassium acetate exhibited after-effect stimulation for a range of low concentrations of the additional compound used in the treatment. Some experiment units whose seedlings had been treated with normal butyric acid or sulphuric acid gave average growth indices that indicate after-effect stimulation but the evidence for this is only suggestive. The recovery-period graphs for all series of modified nutrient solution are drawn to indicate a region of stimulation at the left, above the 100-line, but the actual data are shown in each instance. Their general form is like that of the treatment period graph for the series of simple solutions of acetic acid.

Some attention was given to the toxicity of the distilled water used. Standard seedlings transferred from nutrient solution to distilled water promptly showed growth retardation, which increased as time went on. The toxic action of this distilled water was apparently corrected by the salts in the standard nutrient solution.

Roots in flowing nutrient solution with a gradually increasing additional concentration of acetic acid were studied by means of microscopic measurements made at short intervals. At first the increasing acid concentration produced increasing stimulation of elongation, then decreasing stimulation, then no effect, and finally increasing retardation.

The toxicities of the five compounds added to standard nutrient solution may be compared in various ways. For example, if toxicity is measured in terms of the concentration required to produce a growth retardation of 50 per cent. in the treatment period, the relative toxicities of the five compounds may be estimated as shown on page 442, considering the toxicity of acetic acid as unity.

Some what different scales of toxicity would result if these comparisons were made with respect to other degrees of growth retardation. In gen-

	Concentration producing re- tardation of 50 per cent.	Relative toxicity
	<i>millionths normal</i>	
Acetic acid	1,285	1.00
Propionic acid	550	2.34
Normal butyric acid	280	4.59
Sulphuric acid	550	2.34
Potassium acetate	44,800	0.27

eral it may be said, however, that normal butyric acid was much more toxic than either propionic acid or sulphuric acid, that propionic acid and sulphuric acid were about alike in their toxicity and much more toxic than acetic acid, and that acetic acid was much more toxic than potassium acetate, which was the least toxic of all the five compounds studied. For the three aliphatic acids toxicity is apparently more pronounced as the number of carbon atoms in the molecule is greater.

Stimulation, as an after-effect occurring in the recovery period, might amount to as much as 10 or 20 per cent. It was greatest for potassium acetate, somewhat less for acetic acid and still less for propionic acid. It was suggested for normal butyric acid and sulphuric acid but the evidence for stimulation by these two acids is not in itself very significant, because of unexplained deviations in the average growth indices. The concentration limit below which after-effect stimulation was shown or suggested differed for the different compounds added to standard nutrient solution, as set forth below. These values are in terms of millionths normal and the interrogation points refer to the questionable instances already mentioned.

Acetic acid	920
Propionic acid	210
Normal butyric acid	100(?)
Sulphuric acid	110(?)
Potassium acetate	4,470

For modified nutrient solutions with about these critical concentrations of the respective additional compounds, no effect at all was shown in the recovery period, but these solutions all produced marked growth retardation in the treatment period. For modified nutrient solutions with *lower* concentrations of the additional compounds than those just given, after-effect stimulation was generally shown, or was at least suggested, but the direct effect (in the treatment period) was more or less pronounced retardation—excepting the narrow range of acetic-acid concentrations that produced practically no direct effect and the very narrow range of lowest propionic-acid concentrations that may have produced some direct stimulation.

The five series of experiment solutions with the nutrient salts present are compared with respect to hydrogen-ion concentration (expressed as pH) as well as with respect to concentrations of the additional compounds and physiological effects. With the possible exception of the sulphuric-acid series, it appears that the pH value cannot generally be considered as an index of toxicity, but it is also indicated that hydrogen-ion concentration was markedly influential as one of several or many conditions determining the toxicity of the most toxic solutions. Other solution characteristics—perhaps concentration of anions or of undissociated molecules—were apparently predominant in determining toxicity for the lower concentrations of the organic acids.

Many other interesting relations are shown or suggested by the tables and graphs and some of them are discussed. Influence of the time factor receives some attention and the possibility of synergistic influences in such complex solutions as these is emphasized.

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LIGHT SOURCES AND LIGHT MEASUREMENTS¹

HARDY L. SHIRLEY

Light sources

Plants have developed since their first appearance upon the earth in the earliest geological ages up to the present time under the influence of solar radiation. Until now man has failed to develop any artificial source of light which is equivalent to sunlight in its effects on plant growth. Hence, if we consider plants grown in daylight as normal we must admit that plants grown in artificial light are abnormal in some respects. Therefore, results of experiments with plants grown in artificial illumination will not, in general, be interchangeable with results of similar experiments obtained with plants grown in sunlight. Consequently all experiments designed to study plant growth as it occurs in natural habitats should make use of sunlight if practicable.

SUN

Characteristics of the sun as a source of radiant energy are described by ABBOT (3). Sunlight varies in daily duration from season to season. Its intensity varies from minute to minute with the elevation of the sun above the horizon. It varies from second to second in both intensity and quality, depending upon the prevalence of clouds, smoke, dust and other particles in the atmosphere. The total radiation received at the earth's surface on a cloudy day may be as low as 4 per cent. of that received on a bright day during the same season. The magnitude of these variations can well be appreciated by a study of Weather Bureau records (KIMBALL 38, 39, 40 and 41) and reports of the New York Meteorological Observatory (62).

The spectral energy distribution of many sources of radiation approximates very closely that given off by a black body when heated to the appropriate temperature. To approximate direct radiation from the sun, as received at the surface of the earth, a black body would need to be heated to about 4,800 to 5,300 degrees absolute. This figure is called the color temperature of sunlight. The color temperature of diffuse radiation from the sky or skylight may be as high as 24,000 degrees absolute, or expressed

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differently, about 35 to 50 per cent. of the energy of sunlight is in the visible and ultra-violet region, while the energy of skylight is practically all in this region (PRIEST (68), COBLENTZ, DORCAS, and HUGHES (21), COBLENTZ and KAHLER (22), KIMBALL (42), and MARVIN and KIMBALL (55)). The color temperature of both sunlight and skylight decreases as the sun approaches the horizon. On cloudless days, near noon, skylight comprises about 10 to 15 per cent. of total solar radiation. On overcast days skylight forms 90 to 100 per cent. of total solar radiation. A passing cloud, which obscures the sun, causes a pronounced change in the quality of light by increasing the relative importance of skylight. Likewise light in the shade of trees and buildings is much bluer than direct sunlight. The ever-changing nature of solar radiation, in both intensity and quality, renders it entirely unsatisfactory for use in experiments requiring controlled light conditions. The chief advantages of sunlight are that it produces a more normal form of plant growth than can be produced by any artificial light now available, and that it does not have to be purchased at a high cost.

ARTIFICIAL SOURCES

The most satisfactory artificial light now available is the ordinary Mazda lamp. Mazda lamps are easily obtainable anywhere and are fairly uniform in the quality of radiation they emit. The ordinary tungsten filament lamp with an unfrosted bulb has about 3 to 4 per cent. of its energy in the visible region or a color temperature of about 3,000 degrees absolute. (PRIEST (67), COBLENTZ (19), COBLENTZ, DORCAS and HUGHES (21)), and maintains its quality of radiation fairly uniformly up to 1,000 hours use. The intensity is not constant but is subject to fairly accurate control. Such lamps are the cheapest of all electric lamps and have been used a great deal by many investigators. When used to supplement daylight these lamps are highly satisfactory and are undoubtedly the best now on the market, everything considered. To obtain intensities comparable to those of sunlight a large battery of heavy lamps is required, (ARTHUR, GUTHRIE and NEWELL (7), DAVIS and HOAGLAND (24), HARVEY (33, 34, 35), and HENDRICKS and HARVEY (36)). The writer (78) measured an illumination of 800 foot candles at a distance of 3.5 feet below two 1,500 watt lamps equipped with reflectors. The illumination of sunlight at noon on cloudless days is approximately ten times as high. Not all plants, however, can be grown continuously under illumination from tungsten filament lamps without injury, due apparently to the low intensity in the blue region, (ARTHUR, GUTHRIE and NEWELL (7)).

The white flame carbon arc gives a light having about 25 per cent. of its energy in the visible and ultra-violet region, or a color temperature of

about 3,800 degrees absolute. It tends to reduce the injury to plants noticed with using the tungsten filament bulbs, if the ultra-violet shorter than $290\text{ m}\mu$ is screened out by glass filters, (PRIEST (67), COBLENTZ, DORCAS and HUGHES (21), ARTHUR, GUTHRIE and NEWELL (7)). These lamps are very troublesome to maintain in continuous operation if used without a glass cover as the carbons oxidize rapidly, while if used with the glass cover the glass soon becomes coated with a white deposit which greatly reduces their efficiency.

The mercury arc gives a spectrum very rich in blue and ultra-violet. If mounted in a glass tube this lamp may be used to supplement the light from ordinary incandescent filaments with some success. The spectrum is not continuous, however, and when used alone the lamp is of too low intensity to be of much value in growing plants. The tungsten filament lamp has been combined with the mercury arc in the new General Electric Sun Lamp described by LUCKIESH (52, 53). This lamp is mounted in a special glass bulb which transmits a high percentage of ultra-violet out to the limit of sunlight, wave length $290\text{ m}\mu$. The extreme ultra-violet limit is usually at wave length $253.7\text{ m}\mu$, at which the output is 0.001 per cent. of the total energy radiated. The output at $265.4\text{ m}\mu$ is 0.029 per cent., TAYLOR (82), FORSYTHE, BARNES and EASLEY (26). According to J. M. ARTHUR (personal communication) this lamp will produce typical ultra-violet injury to tomato plants in 90 minutes continuous exposure, being somewhat more severe than the injury produced by irradiation from a mercury arc in quartz through filter "C." (ARTHUR and NEWELL (6)). To insure against injury a filter absorbing all radiation shorter than $290\text{ m}\mu$ should be placed between this lamp and the plant. The lamp is somewhat more efficient in the visible region than the ordinary incandescent lamp but is both more expensive and of shorter life. As constructed at present the sun-lamp cannot be recommended for general use by the plant worker.

For specific investigations in which sources rich in ultra-violet are required the mercury arc in quartz may be used, (COBLENTZ and KAHLER (22) and COBLENTZ, DORCAS and HUGHES (21)). The iron arc is also rich in ultra-violet and presents a more uniform spectrum. Where a continuous spectrum rich in ultra-violet is required for spectrophotometry the under-water spark is more satisfactory, (McNICHOLAS (59)). The neon lamp might prove of value for certain specific investigations where intermittent light of high frequency is desired and where low intensities in the blue region are not a drawback.

Combustion lamps, such as gas, gasoline or kerosene, are unacceptable for ordinary use in plant investigations because of the danger of the unoxidized gases causing severe injury to the plants. Most of them are low

in color temperature and low in intensity. They have been used some in the past for special investigations and may find some limited uses in the future, (BLACKMAN and SMITH (12)).

LIGHT FILTERS

For studying the effects of definite ranges of wave lengths on plant growth light filters are useful. General information on absorption spectra of various dyes and solutions is given by MEES (60) and UHLER and WOOD (83). Infra-red radiation is quite effectively removed by a one centimeter cell filled with a 5 per cent. solution of copper sulphate. Glass filters of high purity are manufactured by the Corning Glass Works, but are expensive. These filters have been used by POPP (66), SAYRE (73), SHIRLEY (78), GRASOVSKY (32) and other workers. Transmission curves for these and other filters are given by GIBSON, McNICHOLAS and TYNDALL (30), GAGE (27) and JONES (37). These filters may also be used with thermopiles and photoelectric cells for measuring definite spectral regions.

GLASSES TRANSMITTING ULTRA-VIOLET

A number of glasses and glass substitutes for transmitting ultra-violet radiation are on the market. Such glasses have been used by plant workers as filters with the quartz mercury arc and for transmitting the ultra-violet of sunlight. Many of the glasses change their transmission with use, especially when subjected to the quartz mercury arc. Data on the transmission of various glasses are given in the following publications: ARTHUR and NEWELL (6), U. S. Bureau of Standards Circular letter 235 (84), COBLENTZ and STAIR (23), GIBSON, McNICHOLAS and TYNDALL (29). These glasses are being recommended by their makers for use in phototherapy and are constantly being changed. COBLENTZ and STAIR (23) list them according to transmission at 302 m μ as follows:

Transmission at 302 m μ

Less than 1 per cent.	Common window glass, Quartz-lite
About 25 per cent.	Vita-glass, Sanalux, and Renovic
About 35 to 40 per cent.	Holviglass, Sunlit, and Sendlingers U-V glass
About 45 to 50 per cent.	Helioglass, Uviol-Jena, Neuglas
About 60 to 65 per cent.	Corex-D
About 80 to 90 per cent.	Corex-G981FF ² and Quartz glass

Light measurements

The methods used in measuring light will vary with the source of light used and the specific purpose of the investigation. For measuring ultra-violet radiation, lithium or uranium photoelectric cells may be used; for measuring visible radiation illuminometers are best suited, while for measuring total radiation non-selective radiometers are required.

Where a study of the effects of specific wave lengths is not the subject of an investigation certain general requirements may be laid down for the type of the light measuring device to be used. In ecological work and for most work in plant physiology a light measuring device should satisfy the following requirements:

1. It should be uniformly sensitive to the visible region of the spectrum. Sensitivity to the infra-red is less objectionable than sensitivity confined mostly to the blue and violet regions.
2. It should admit of standardization so that the readings may be expressed in units widely understood.
3. It must cover a range of intensities from 10 to 10,000 foot candles, or from 0.001 to 1.3 gram calories per square centimeter per minute.
4. It should be easy to make the readings and simple to convert them to standard units.
5. It should be rugged enough to withstand handling in the greenhouse or field.

METHODS AVAILABLE

Light is known to us only by its effects. In measuring light we may measure one of its several effects. These may be divided into two groups depending on whether the effect is selective, that is, confined to certain wave lengths only, or whether it is non-selective, occurring uniformly throughout the solar spectrum. For convenience in discussion we will consider the following effects:

Non-selective

Heating effects

Selective

Electrical effects

Illuminating effects

Chemical effects

HEATING EFFECTS.—Only those methods which measure the heating effects of light are uniformly sensitive to all wave lengths. These are the methods which have been adopted as standard by the physicists and astronomers throughout the world.

Thermopiles.—If to each end of a short piece of bismuth wire, a piece of silver wire is soldered a thermocouple is formed. When one bismuth-silver junction is heated above the temperature of the other an electromotive force is produced which may be detected by connecting the silver wires to a galvanometer. The magnitude of the electrical potential produced is almost directly proportional to the temperature difference between the junctions. Two or more thermocouples connected in series form a thermopile. Any two dissimilar metals may be used for making a thermopile but

certain ones are most useful. Thermopiles are one of the most widely used instruments for radiation measurements.

KIMBALL and HOBBS (43) designed a pyrhelimeter with a thermopile as the active element, which has been in use as a standard instrument of the United States Weather Bureau since 1923. The hot junctions of the thermopile are in thermal contact with a thin copper ring blackened with lampblack. The cold junctions are in thermal contact with a surrounding concentric ring which is coated with zinc oxide or magnesium oxide. The instrument measures the difference in temperature of these two surfaces when sunlight falls upon them. This instrument is almost uniformly sensitive throughout the visible spectrum but has a low sensitivity to infrared radiation due to the fact that zinc oxide and magnesium oxide have high absorptive capacities for the longer wave lengths. This instrument is now being mounted in an electric lamp blank by the Eppley Laboratory of Newport, Rhode Island. The instrument is somewhat delicate, requires from 1 to 4 minutes to come to equilibrium and gives only a feeble current when exposed to radiation intensities below 0.1 gram calorie per square centimeter per minute. It may be connected to either a recording or direct reading microammeter.

BURNS (15) used a COBLENTZ (18) thermopile connected with a galvanometer circuit, which was balanced by a battery circuit so that no current was drawn from the thermopile at time of reading, for measuring radiation intensity in the forest. Both the COBLENTZ thermopile and the galvanometer are delicate and somewhat difficult to operate in the field by the untrained worker. The thermopile receives radiation from only one direction. For measuring specific wave lengths, the COBLENTZ thermopile, equipped with filters, is quite satisfactory. Because of its construction the COBLENTZ thermopile is more nearly free from zero shift than any other now available. COBLENTZ thermopiles may be had from the Eppley Laboratory of Newport, Rhode Island.

The MOLL (61) thermopile is very rapid in reaction and quite sensitive to moderately low intensities. Types are now made suitable for use in measuring sunlight. BIRGE and JUDAY (10) used a MOLL thermopile for measuring solar radiation under water with quite satisfactory results. Both KLUGH (46) and the writer have found them to be unsatisfactory for measuring the lower intensities such as occur in forests or in greenhouses on cloudy days. Such thermopiles also have a zero shift.

GORCZYNSKII (31) used a MOLL thermopile with a clock driven equatorial mounting for measuring solar radiation in the deserts of Africa. The thermopile was connected to a Richard recording millivoltmeter.

BIRGE (9) used a silver-bismuth thermopile designed by C. E. MENDEN-

HALL, University of Wisconsin, for measuring radiation in inland lakes. SHELFORD (75) has also used this thermopile.

GAST (28) constructed a thermopile in which the receptors are 5 silver spheres blackened with lampblack. The active elements are constantan and iron. The thermopile is mounted inside of an evacuated lamp form and is sufficiently rugged for ordinary field handling. It has a certain zero shift or lag, and when used with ordinary microammeters is rather insensitive to low radiation intensities. GAST attempted to build a radiometer which would be uniformly sensitive to radiation regardless of the direction from which it comes. For perfect performance his receptor should be a single sphere of uniform sensitivity located in the exact center of the spherical bulb. As ordinarily used, three or more thermopile units are mounted in series at one station and connected to a recording microammeter. By using several units at one station a more precise sample of the intensities over an area is obtained than would be by a single thermopile.

A modified form of the constantan-silver thermopile developed by WILSON (86) has been used by SHIRLEY (79) for measuring light in a greenhouse and in the forest. This thermopile is exceedingly rugged and quite sensitive to radiation intensities below 0.1 gram calorie per square centimeter per minute, as well as to the highest intensities encountered in sunlight. This thermopile was not injured by frequent use in the forests of northern Minnesota from June until October. As at present constructed, however, the thermopile is subject to a zero shift which must be corrected for in making readings.

All the thermopiles mentioned above may be used as direct reading instruments when connected with a galvanometer or microammeter. They may also be used for recording radiation from one or several stations. Weather Bureau, MOLL, GAST and SHIRLEY thermopiles all give sufficient current to be used with a recording microammeter of the ENGELHARD type or a recording potentiometer of the Leeds Northrup type. RICHARD recording millivoltmeters and recorders of German make are less expensive but do not possess the sturdy construction or reliability of the above-named American instruments.

Solar radiation records obtained from thermopiles or resistance thermometers (see below) may be used for many purposes. They give the maximum intensities and show the variations of intensity from time to time. If the area under the curve is integrated a measure is available of the total radiation received during a definite period of time. An instrument for integrating solar radiation is on the market composed of a MOLL thermopile connected to a gas microcoulomb meter. The writer is unable to get any definite information on the performance of these instruments.

The Smithsonian pyranometer (ABBOT, 4) is one of the most precisely made instruments for the measurement of total solar radiation or radiation from the sky. It consists of a thermopile, the cold junctions of which are in contact with a heavy copper disk, while the hot junctions are in contact with a blackened manganin receptor. The amount of deflection of an undamped galvanometer caused by a 3-second exposure to radiation is read. A current of known strength is then sent through the receptor for 3 seconds which will give approximately the same deflection of the galvanometer. By calculating the heating value of this current, the heating value of the radiation absorbed can be found and expressed directly in heat units. The writer is informed by Dr. C. G. ABBOT that these instruments have given satisfactory performance when in daily use for over 15 years at Smithsonian Observatories. They are fairly rugged and can be used in the field if moderate precautions are taken. They are accurate to within about one per cent. over a range of intensities varying from 1 to 100.

The Smithsonian pyranometer is a modification of the ÅNGSTRÖM pyrliometer which was a favorite with solar observers in the past, ABBOT (3).

RESISTANCE THERMOMETERS.—Practically all electrical conductors change in resistance with change of temperature. This principle is made use of in the construction of resistance thermometers and pyrliometers.

The MARVIN silver disk pyrliometer, FOOTE (25), of the United States Weather Bureau is of this type. A fine platinum wire is placed inside a blackened silver disk which is carefully shielded from heat changes in the environment. The whole is mounted equatorially and driven by a clock. The amount of radiation falling on the disk is determined by measuring the change in resistance of the wire when the disk is exposed. Such instruments are ill adapted for field use due to the complexity and size of the apparatus and the difficulty in making accurate resistance measurements in the field.

The Smithsonian silver disk pyrliometer, ABBOT (2, 3), consists of a mercurial thermometer, with the bulb mounted inside a silver disk. Mercury contact is maintained between the disk and the bulb. The whole is carefully shielded from variations in air temperature by mounting inside a wooden block. The difference between the thermometer readings before and during exposure to radiation gives a measure of the amount of radiant energy received. The instrument is sufficiently rugged and portable to be used in the field; however, it is not sensitive to low radiation values.

The ABBOT (1) water flow pyrliometer is an accurate instrument. It measures the radiation intensity by measuring the increase in temperature of water as it flows at a uniform rate around a cylinder exposed to radia-

tion. The instrument is not suitable for field use since it requires elaborate equipment for maintaining the water at constant temperature. Even with the modification suggested by SHULGIN (80) it would hardly be practical for use by the plant worker.

All instruments described above may be standardized to express radiation regardless of quality, in gram calories per square centimeter per minute. Those to be described hereafter cannot be so standardized. Practically all these instruments may be used behind filters for measuring specific spectral regions.

The sunshine recorder, MARVIN (54), consists of a black and white bulb gas thermometer connected together with a U-tube partially filled with mercury. When the sun is shining, the increased pressure of the gas in the black bulb forces the mercury up the U-tube where it completes an electric circuit connected with a recording device. These records show merely how many hours the sun shone on the recorder during any interval of time. They make no measure of intensity.

Radiometers consisting of vanes mounted on a rotating axis with one side blackened and the other polished are sensitive to direct radiation but are not suited for use in diffuse light. They are scarcely practicable for use in plant work.

Radio-atmometers consisting of paired black and white atmometers, recommended by LIVINGSTON (51), may be used to give rough estimates of the radiant energy available. The writer found them to give unsatisfactory results when used in diffuse light of low intensity. They are probably of considerable value for use as integrating devices in stations which can be visited only once a week or so, BURNS (14).

ELECTRICAL EFFECTS.—When light impinges on thin films of metals which are mounted in a bulb and given a negative charge, these films lose their charge. Such a bulb constitutes a photoelectric cell. Lithium, sodium, potassium, rubidium, caesium and other metals have been used in making photoelectric cells. These metals show increasing sensitivity to the longer wave lengths as their atomic weight increases. The sensitivity of lithium cells is confined almost entirely to the ultra-violet region, while caesium cells are sensitive to the red and infra-red as well as the violet.

Photoelectric cells are practically instantaneous in speed of reaction. Utilization of this property has made possible movietone pictures and television.

Photoelectric cells which maintain their sensitivity constants for considerable periods of time are now being made quite cheaply. When used with monochromatic light, or light of constant color temperature, they are excellent instruments. Photoelectric cells have been made which have

almost as great sensitivity to light as the human eye. The photoelectric current produced is directly proportional to the radiation intensity received over considerable ranges of wave length. The current may be measured directly by a microammeter, or it may be amplified by vacuum tubes and the amplified current measured.

Photoelectric cells may also be used with recording galvanometers similar to the types used for thermopiles. In addition photoelectric cells may be connected with a condenser and glow relay tube for recording purposes, as explained by RENTSCHLER (70) and POOLE (65).

A plant investigator would ordinarily want to use a cell which is sensitive to all radiations of visible light. The caesium, calcium or strontium cells are the most satisfactory in meeting these requirements at present. The cell is used with a 20 to 90 volt "C" battery and a microammeter. The instrument is sufficiently rugged and compact for ordinary field use. While a photoelectric cell is not equally sensitive to all wave lengths sensitivity curves may be worked out so that when used with filters it makes a fairly satisfactory light measuring device.

Photoelectric cells have certain characteristics which render their use by beginners difficult: 1. The current given is directly proportional to the radiation intensity only within definite limits. 2. The current given varies with the impressed voltage for constant illumination. 3. Many cells show fatigue—a decreasing current with increase in exposure time. 4. Most commercial cells have concave sensitive surfaces which are poorly suited for measuring radiation from more than one direction. 5. Cells are liable to ionization injury if too high voltage is used when exposed to high light intensities. 6. Each cell has a special sensitivity curve at different wave lengths which can only be determined by direct test. This renders standardization very complex. 7. Infra-red radiation causes a decrease in the current given by a given intensity of variable radiation which may amount to as much as 50 per cent., OLPIN (64).

Workers contemplating the use of photoelectric cells should consult COBLENTZ (20), SHELFORD (75 and 76), SHELFORD and KUNZ (77), KUNZ and SHELFORD (48). The measurement of sunlight with photoelectric cells is a far more complicated process than would be inferred from papers by some workers, SEGELKEN (74).

The selenium cell changes its resistance upon exposure to light. It is not equally sensitive to different wave lengths and also has a temperature error. Due to the difficulty of measuring resistances accurately in the field, this cell has little to offer the plant investigator, COBLENTZ (20).

ILLUMINATING EFFECTS.—The most sensitive instrument to light which we know of at present is the animal eye. The human eye can read ordinary

news print over a range of intensities varying from 0.004 to 10,000 foot candles, or from one to two and one-half million. Because of its adaptability to such a great range it is relatively insensitive to small changes in intensity. The human eye so rapidly adjusts itself to the intensity of illumination that estimates based on the eye alone are practically valueless.

The Macbeth illuminometer, Leeds and Northrup Company (50), is a very satisfactory instrument for measuring light when the color temperature of the source is the same as that of a standard lamp. By using neutral filters it covers an extremely wide range of intensities, being limited only by the limitations of human vision. The instrument is quite rugged and well adapted for field use. The intensity of light reflected from a test plate is compared with the intensity of light from a working standard lamp, which may be moved nearer or farther from the eye as required to obtain an intensity match. Readings are made in foot candles directly. Considerable difficulty is encountered in using this instrument to measure sunlight because of the differences in color. This may be partially compensated for by the use of a color filter; however, even by using filters two observers may differ as much as 10 per cent. on estimates. For measuring the illuminating intensity of artificial light the Macbeth illuminometer is probably the best instrument available. A sensitivity curve for the eye is given by KIMBALL (38).

Since thermopiles measure the heating effect, and illuminometers the lighting effect of radiation, measurements made by the two instruments are not directly comparable unless the source has a constant color temperature. Last summer the writer made a series of simultaneous readings with the Macbeth illuminometer and the SHIRLEY pyrliometer under Norway pine canopies. The ratios of the two readings were averaged and standard deviation calculated. The standard deviation of a single determination was often as much as 25 per cent. of the ratio, as determined from 50 values. When readings in the forest were expressed as percentage of radiation in the open as measured by the same instrument, quite comparable results were obtained.

Extinction photometers that make use of a neutral wedge which gradually cuts off more and more light until none passes through it may be used as rough light measuring devices. Such instruments do not measure light in absolute units. The value obtained will vary greatly with the observer and acuity of his vision. These photometers are rugged, simple to use, and may be carried in the pocket but cannot be recommended for general use in plant investigations.

Spectrophotometers have been used to measure the intensity and quality of light in the forest by KNUCHEL (47) and KLUGH (46). KNUCHEL

(47) used an instrument of the standard type. It was equipped with an electric lamp to serve as a standard source of radiation. This instrument is very valuable for special investigations in which quality changes are important; however, it is too delicate and cumbersome for general field use.

The pocket spectrophotometer of NUTTING (63) makes use of the extinction principle. It was used by KLUGH (46) for making relative measures of different spectral regions. It can be used for determining changes in light quality in general but is not a precision instrument.

CHEMICAL EFFECTS.—Most of the light measurements made by plant workers in the past have been made with various kinds of chemical photometers. Practically all of these photometers make use of the darkening of silver salts when exposed to light. A number of devices have been constructed for exposing the sensitized paper or plates, many of which are described by ZON and GRAVES (87), PULLING (69), KLUGH (46), RUBEL (72) and ABDERHALDEN (5). CLEMENT's photometer is about as satisfactory as any. The essentials of the method are: a piece of the sensitized paper or plate is exposed to the light for a definite period of time, or until it attains a certain definite tint. The light intensity is assumed to be inversely proportional to the length of time it takes the sensitized material to reach the standard tint.

The method has several defects. Practically all sensitized materials used are unequally sensitive to the different wave lengths, being most sensitive to the blue and ultra-violet and only slightly sensitive to the red. Panchromatic plates as used by KLUGH (45) are fairly uniformly sensitive throughout the visible region but are highly sensitive to the ultra-violet. Panchromatic plates must be developed before the tints can be compared, in which process standard methods must be used if accurate results are to be obtained. The plates also vary greatly in sensitivity. Rhodamin B paper is less accurate but is sensitive to red. In no case is the time required to attain a standard tint exactly inversely proportional to the light intensity; however, correction factors have been worked out for a number of plates. In intense light, the time required to attain a standard tint is often so short that it cannot be accurately measured even with an automatic stop watch arrangement as used in CLEMENT's photometer. It is very difficult to reduce the readings to standard units so that the intensities in one place may be compared with those of another. A recent improvement on the CLEMENT's photometer adapts it for recording purposes.

Other chemical reactions have been used in evaluating light. The decomposition of hydriodic acid may be used to integrate the light over a considerable period of time. They present the same general difficulties mentioned above, RIDGEWAY (71) and McCREA (56).

In general the method has practically all the objections open to the photoelectric cell without having the speed and accuracy of the latter instrument. Foresters and ecologists seem to be gradually adopting thermopiles, photoelectric cells, and illuminometers to replace chemical photometers in light measurement work, especially in studies requiring rather precise measurements. Chemical photometers may still have valuable uses where only rough approximations are needed. The chief advantages are compactness, portability, and inexpensiveness.

For a discussion of photochemical processes the reader is referred to KISTIAKOWSKY (44).

PLANT INDICATORS.—Phytometers would serve as an admirable method of measuring the growth value of light provided suitable plants are found whose light reaction is sufficiently sensitive and accurately measurable. CLEMENTS and GOLDSMITH (16) give a discussion of the method and some results obtained with sunflowers. Since the reaction of a plant to light is greatly disturbed by temperature and other conditions, it is doubtful if phytometers can be successfully employed without measurements by physical instruments as checks. The indicator value of natural vegetation has not been sufficiently studied to be used with assurance at present. As an indicator of light conditions certain broad differences in intensities can be detected by this means, but, for fine distinctions, physical instruments would have to be relied upon.

Measurement of transmission, reflection and absorption of leaves

Various workers have attempted in the past to measure the efficiency of different wave lengths of light in plant photosynthesis. In such experiments it is imperative to know how much energy was actually absorbed by the assimilating organ. SHULL (81) has shown that the reflection from leaf surfaces is selective, i.e., green is most highly reflected while red and blue are reflected to a much less extent. The precise measurement of the transmission, reflection and absorption of diffusing surfaces is a very difficult problem. Those interested are referred to McNICHOLAS (57, 58), BLOCK and PIRANI (13), LAX, PIRANI and SCHÖNBORN (49), and WALDRAM (85). Such problems are in general too technical to be undertaken by the ordinary plant investigator without the aid of a competent physicist. The reader is further referred to methods in use at the Electrical Testing Laboratories of New York and the United States Bureau of Standards, Washington, D. C.

PHOTOMETRIC DEFINITIONS

The following photometric definitions are essentially as given by the committee on nomenclature and standards in the *Transactions* of the Illuminating Engineering Society 25: 728-747, October 1930.

1. **Light:** For the purposes of illuminating engineering, light is radiant energy evaluated in proportion to the luminous sensation produced by it.

2. **Radiant Flux:** Radiant flux is the time rate of flow of radiant energy. It is expressed preferably in ergs per second or in watts.

3. **Luminous Flux:** Luminous flux is the time rate of flow of light.

4. **Lumen:** The lumen is the unit of luminous flux. It is equal to the flux in a unit solid angle (steradian) from a uniform point source of one candle, or to the flux on a unit surface all points of which are at unit distance from a uniform point source of one candle.

5. **Luminous Intensity:** Luminous intensity, in a given direction, is the solid-angular flux density in the direction in question. Hence, it is the luminous flux on a small surface normal to that direction, divided by the solid angle (in steradians) which the surface subtends at the source of light.

6. **Candle:** The candle is the unit of luminous intensity. It was originally based on the amount of light given by a standard sperm candle burning under fixed conditions. It is now standardized and used as an international unit.

7. **Candle-power:** Candle-power is luminous intensity expressed in candles. In addition to the standard candle there are certain standard lamps which must be burned under stated conditions:

Standard Pentane Lamp burning pentane gives 10.0 candle-power

Standard Hefner Lamp burning amyl acetate gives 0.9 candle-power

Standard Carcel Lamp burning colza oil gives 9.6 candle-power.

8. **Illumination:** Illumination is the density of the luminous flux on a surface, or the quotient of the flux by the area of the surface when the latter is uniformly illuminated.

9. **Foot-candle:** The foot-candle is the unit of illumination when the foot is taken as the unit of length. It is the illumination on a surface one square foot in area on which there is a uniformly distributed flux of one lumen, or the illumination produced at a surface all points of which are at a distance of one foot from a uniform point source of one candle.

10. **Lux:** The lux is the practical unit of illumination in the metric system, equivalent to the "meter-candle." It is the illumination on a surface one square meter in area on which there is a uniformly distributed flux of one lumen, or the illumination produced at a surface all points of which are at a distance of one meter from a uniform point source of one candle.

11. **Phot:** The phot is the unit of illumination when the centimeter is taken as the unit of length; it is equal to one lumen per square centimeter.

Foot-candle equals 10.764, Lux equals 1.0764 milliphot.

12. **Brightness:** Brightness is the quotient of the luminous intensity of a surface measured in a given direction by the area of this surface projected on a plane perpendicular to the direction considered.

13. **Lambert:** The lambert is a unit of brightness equal to the average brightness of any surface emitting or reflecting light at the rate of one lumen per square centimeter, or the uniform brightness of a perfectly diffusing surface emitting or reflecting light at that rate.

For most purposes the millilambert, 0.001 lambert, is the preferable practical unit.

14. **Foot-Lambert:** The foot-lambert is a unit of brightness equal to the average brightness of any surface emitting or reflecting light at the rate of one lumen per square foot, or the uniform brightness of a perfectly diffusing surface emitting or reflecting light at that rate.

MECHANICAL EQUIVALENT OF LIGHT

For radiation of maximum visibility one lumen equals 0.001496 watts, or one watt radiation of wave length 556 equals 668 lumens.

For sunlight one calorie per square centimeter per minute is the equivalent of 5,000–7,000 foot candles.

Tungsten filament lamps give much lower values.

Summary and conclusions

A critical discussion of light sources available to plant workers is given.

A discussion of the characteristics of various thermopiles, pyrhelimeters, photoelectric cells, illuminometers and chemical photometers is given.

The ordinary Mazda lamp, with or without filters, is the most satisfactory source of light for general use in plant investigations.

The General Electric Sunlamp as now made is not practical for use in growing plants.

Thermopiles and pyrhelimeters, with or without filters, may be successfully employed by the plant worker for measuring light, and are to be preferred in general to photoelectric cells, illuminometers and various chemical photometers.

The writer wishes to acknowledge the kindness of Dr. H. H. KIMBALL and Dr. C. G. ABBOT in showing him the instruments used by the United States Weather Bureau and the Smithsonian Institution, respectively. The writer is also indebted to members of the United States Bureau of Standards, the Edison Lamp Works of Harrison, New Jersey, and the Electrical Testing Laboratories of New York, for explaining methods used in radiation investigations.

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SOME EFFECTS OF ACETYLENE ON THE RIPENING PROCESSES OF BANANAS¹

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(WITH EIGHT FIGURES)

Introduction

In recent years there has been considerable in the literature concerning the action of ethylene and propylene on the ripening processes of fruits. Under certain conditions, it would appear that both of these gases are capable of hastening the ripening of some fruits, DENNY (3, 4); REGEIMBAL (28); ROSA (30, 31); and OVERHOLSER (26). Whether the action of these gases is due to the fact that they are unsaturated compounds or to some other property common to both remains yet to be shown. However, acetylene, another hydrocarbon which is even more unsaturated than the aforementioned ones, has been little experimented with in this regard. HARVEY (14) reports it as being unsatisfactory, but does not present experimental evidence to indicate the nature of its action. Any information concerning its action on the ripening processes of fruits would be of interest therefore, first, because it would be new regardless of whether or not the action were similar to that of ethylene and propylene; and secondly, if similar, it would tend to strengthen the hypothesis that the action of these gases is in some way associated with the unsaturated condition of the molecule.

It is the purpose of this paper to present the results of experimentation on the effects of acetylene on the ripening processes of both normal and chilled bananas. It is not considered that this is a finished piece of work, in any sense; but it is hoped that it may be of interest or suggestive to those now engaged in projects along these lines. The work reported was done in 1928.

Materials and methods

The fruit used in the first experiments was received from the United Fruit Company.² It was shipped by express from New York City to Ithaca, N. Y., arriving there about 24 hours after unloading in New York. It was the Gros Michel variety and was green at the beginning of the experiments unless otherwise stated.

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² The work herein reported was started during the tenure of a United Fruit Company Fellowship. It was done in the Laboratory of Plant Physiology, Cornell University.

The green fruit is very hard. The hardness was measured in these experiments by a specially devised instrument which measured the force required to insert a cylindrical metal plunger a given distance into the pulp. In these results, the index 7 indicates a very hard, green fruit; whereas 1 or less indicates a very soft, or ripe fruit.

The starch content of the green fruit is very high, a cross-section of the fruit becoming entirely black when treated with a few drops of iodine-potassium iodide solution. In the ripe fruit, the starch has largely or completely disappeared as indicated by an absence of coloration with the aforementioned test. Generally, the ripe fruit still shows starch in the middle and in three narrow bands radiating from the middle of the pulp. These areas, however, are not as black as in the green fruit, and the intermediate areas may be completely free of any starch.

All samples were composed of a number of individual fruits which were selected to be as comparable as possible. An individual fruit is called a finger and a cluster of these a hand. In the main experiments, the control and treated samples were composed of halves of the same hands.

The temperatures, though fluctuating in some instances, were alike in all cases in both control and treated samples. In the respiration experiments the jars containing the fruit were submerged in controlled temperature baths. The air was brought to temperature before its entrance into the chambers.

The only differences, therefore, between the treated and the controls was the presence of the acetylene or carbide. The humidity was high in all cases as indicated by the condensation of moisture on the inner surfaces of the containers.

In the jar experiments, carbide was placed in the bottom of 3.5 liter battery jars and covered with cotton to prevent contact with the fruit. The tops of the jars were covered with a layer of cotton to allow air exchange while still preventing a too rapid loss of acetylene and moisture.

Respiratory rate was determined by use of an apparatus which permitted a continuous flow of CO_2 -free air. The determinations were made over two hour periods by the absorption of CO_2 in NaOH by means of modified Reiset tubes. The residual alkali was titrated with standardized HCl after the addition of BaCl_2 to precipitate the carbonate. The respiratory rate was calculated as milligrams of CO_2 per kilogram of fruit per hour, ($\text{CO}_2\text{-MKH}$).

Preliminary experiments

The results of a couple of experiments will serve to illustrate the results obtained in several preliminary tests.

EXPERIMENT I

On March 21, 1928, eight firm, green fingers were placed in each of two battery jars, (3.5 liters capacity). About 12 grams of calcium carbide were placed in the bottom of one. Each jar was covered with a layer of cotton. The temperature during the experiment varied from 16 to 24° C. (61–75° F.). The treated fruit began to turn yellow in three days, the control fruit remaining green. At the end of five days the treated fruit was ripe. It had a good yellow color, medium starch content, and a fairly good flavor; whereas the check fruit was just beginning to assume a yellow color, had a high starch content, and was still inedible.

This experiment was repeated a number of times with different lots of fruit and in no case, when the fruit used was green at the beginning and the temperatures maintained within this range, did the control fruit ripen as quickly or as uniformly as the treated. In those instances in which the fruit had clearly begun to ripen or where temperatures much over 24° C. (75° F.), were encountered for any length of time the differences were questionable though always favoring the treated as regards the color of the peel. On the other hand, in a few cases in which the treated fruit ripened well in five days, the check samples were still green at the end of twelve days.

EXPERIMENT II

An experiment was planned to show the relative efficacy of varying amounts of carbide. As all the amounts used were equally effective, the experiment merely indicates the difference which may be obtained due to the treatment of bananas with carbide. Portions of hands, composed of seven or eight fingers, were placed in jars covered with cotton. There were about 2.5 liters of air space remaining in the jars after the fruit was placed therein. Amounts of carbide varying from 0.25 to 4.0 grams were placed in the jars. Cotton was placed on the carbide to prevent contact with the fruit. The jars were placed together in the laboratory. The room temperature varied from 12.2 to 24° C. After the first two days the temperature was not above 20.6° C., and was above 15.6° C., most of the time. The temperatures encountered, therefore, were favorable for ripening but were not sufficiently high to cause its undue acceleration. When the experiment was started, June 13, 1928, the fruit was green in color and showed a pulp pressure of 5.5. The observations made three, and five and one-half days later are presented in table I.

This experiment clearly shows the effect of the carbide treatment, and furthermore, tends to indicate that there are no sharp limits to the effective concentration of acetylene. The results obtained are the more striking in view of the fact that the odor of acetylene was readily detected several feet from the group of jars, including those containing the control fruit.

TABLE I
OBSERVATIONS ON FRUIT IN EXPERIMENT II

GRAMS OF CARBIDE	END OF 3 DAYS				END OF 5.5 DAYS			
	PRES- SURE	STARCH CONTENT	FLAVOR	COLOR*	PRES- SURE	STARCH CONTENT	FLAVOR	COLOR
0.25	1.0	high	very green; very astringent	g-y	0.7	medium	good	golden yellow
0.5	1.0	medium high	slightly green; slightly astringent	y-g	0.5	"	"	"
1.0	1.0	high	green; astringent	g-y	0.5	"	"	"
1.5	0.8	medium high	"	slightly g-y	0.2	"	fair	"
2.0	1.0	"	"	slightly g-y	0.5	"	good	"
3.0	1.0	medium	"	slightly g-y	0.7	low	"	"
4.0	1.0	high	slightly green; slightly astringent	yellow	0.5	medium	"	"
Check 1	3.8	very high		green	1.3	very high	astringent	y-g
Check 2	3.5	very high		green	1.8	very high		green

* g-y greenish yellow.

y-g yellowish green.

The effect on respiration

EXPERIMENT III

Because a preliminary experiment had suggested that respiration was increased during treatment, another trial was made with greener fruit to check the observations. The samples were composed of halves of the same hand and each contained six fingers. The treated sample weighed 1,021 and the control 958 grams at the beginning. The temperatures were high but alike for both samples. The air was drawn through the chambers at as near the same rate as possible by comparing the rate of bubbling. It was approximately 15 liters per hour. The results are presented in figure 1.

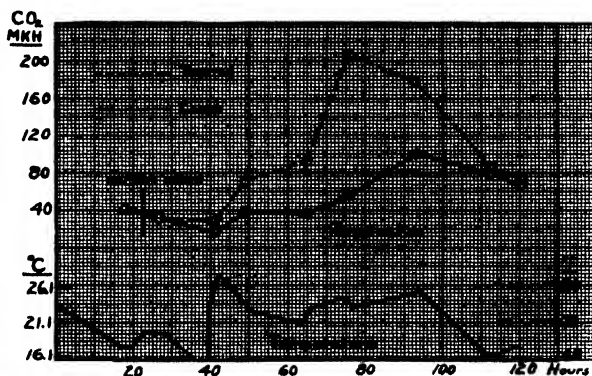


FIG. 1. Effect of the carbide treatment on the respiration of green bananas at room temperature. Exp. III.

At the end of 120 hours, the treated fruit was excellent in appearance, being uniformly golden yellow. It had a very good flavor, medium starch content, and a pressure of only 0.5. The check, at this time, was of variable color, being from yellow to green. It was not ripe as indicated by flavor, high starch content, and a pressure of 1.2.

The treated fruit ripened quicker, better, and more uniformly than the control. Accompanying this there was a marked acceleration of respiration. It was not affected at the first determination, (2.5–4.5 hours), following the beginning of the treatment. The maximum difference occurred at 52.5–54.5 hours after the initiation of the treatment, at which time that of the treated was 3.6 times that of the control.

Effect on respiration of severely chilled fruit

EXPERIMENT IV

The fruit used in this instance had remained packed in barrels with hay for two days after arrival and was light green in color. It had an

index for pressure of 7.0–7.5, indicating that very little if any ripening had occurred though the temperature of the fruit at the end of two days was 21.1° C.

Three hands from the middle of the stem were subjected to temperatures of 4.4–10° C., for 38 hours. Most of the time the temperature was below 7.2° C. The chilling temperatures are shown in table II. At the

TABLE II
TEMPERATURES (°C.) DURING THE CHILLING PERIOD, EXPERIMENT IV

HOURS OF CHILLING	0	1	1.5	2.5	11.5	13.5	20	26	38
Air temperature	12.5	7.8	6.9	6.7	6.9	4.4	7.2	6.7	11.0
Pulp temperature ..	21.0	11.7	8.0	7.2	7.2	4.4	7.2	6.7	10.0

end of this treatment the pressure was 5.5. At this time the hands were cut into halves and two samples made, each composed of halves of the same three hands which had been subjected to identical treatment. The samples were quickly weighed and placed in the respiration chambers. The weights of the two samples were 2,129 grams for the treated, and 2,136 grams for the control. The air flow was regulated to 20 liters per hour as measured by calibrated flow-meters. The temperature was closely maintained at 21° C. The only difference in the two samples was that, in the case of the treated, the air was drawn over 15 grams of calcium carbide immediately before entering the chamber. When first opened, at the end of 77.5 hours, the chambers still smelled strongly of acetylene though most of the carbide appeared to have been used at the end of 24 hours. The results are presented in figure 2. At 128 hours the pressure of the treated was 0.9 whereas that of the control was 2.8. These were essentially the same as the determinations at 77.5 hours. The flavor of the treated was

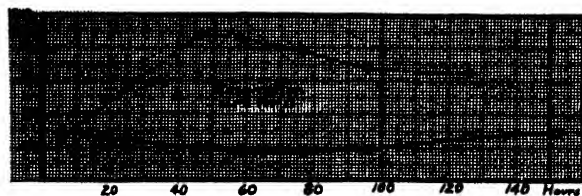


FIG. 2. Effect of carbide treatment on respiratory rate of severely chilled bananas.
Exp. IV. ----- treated
————— control

fairly good at this time but that of the check was not good even at 203 hours. The observations as to color and starch are given in table III.

TABLE III
RESULTS OF EXPERIMENT IV

TREATMENT HOURS AT 21° C.	COLOR		STARCH	
	CONTROL	CARBIDE	CONTROL	CARBIDE
0	yellowish-green	yellowish-green	high	high
59	" "	greenish-yellow	" "	" "
78	" "	yellow	high	medium-high
128	" "	slightly pale yellow (no green tips)	" "	medium-low
203	3 yellow fingers (green tips) most yellowish- green		high	

The results obtained warrant the conclusions that the fruit was severely chilled and that the carbide treatment markedly influenced the rate of ripening as indicated by increased respiratory rate, (at 45-47 hours after the beginning of the treatment, that of the treated was 4.4 times that of the control), more rapid softening, loss of starch, and improvement of color and flavor.

Ammonia, an impurity in acetylene

In view of the work of BACHER (1) which showed that small amounts of ammonia in the air stimulated the growth of certain plants, and also that ammonia is generally conceded to be one of the chief impurities in acetylene, (LEWES 19, p. 472, LIEBETANZ 20, p. 247, and MATHEWS, 22), it seemed well to test the effect of this gas on the ripening of the fruit. In addition to treating the fruit with ammonia, the acetylene-air mixture was also purified by a method suggested by MATHEWS (22) which would remove any ammonia present. The resulting gas-air mixture, which was presumably free of ammonia, phosphorus compounds, sulphides, carbon monoxide, and carbon dioxide was passed through the respiratory chambers. It is quite likely that any possible trace of ethylene would be oxidized by the chromic acid (21, p. 91).

EXPERIMENT V

The purpose of this experiment was to determine whether ammonia was responsible for the effects previously attributed to acetylene. The fruit used was obtained from a local dealer but was still green as shown by appearance, pressure, and the respiration curve of the check. Halves of

three hands were used, and the weight of each sample was in the neighborhood of two kilograms. The procedure was similar to that in the previous experiments. The temperatures were closely maintained at 21° C. The ammonia was supplied as ammonium carbonate, over which air was passed before entering the chamber. It was planned to use an amount of the salt which would give, if uniformly distributed over a 24-hour period with an air flow of 20 liters per hour, a concentration twice that which BACHER found to be best for growing plants. It was not expected that the fruit would respond as readily as growing plants. As the first treatment appeared to be ineffective, two additional treatments of twice the original quantity were made. Comparisons were made with the untreated fruit, fruit given the carbide treatment as previously, and that exposed to the purified gas-air mixture. The results are presented graphically in fig. 3.

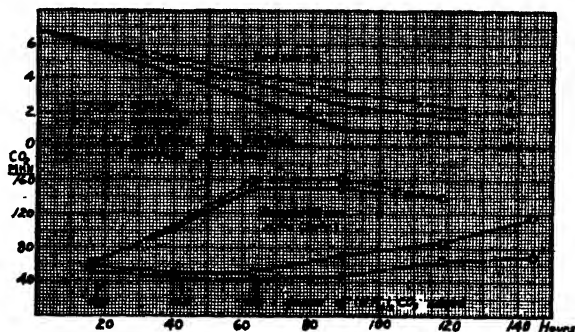


Fig. 3. Effect of ammonia, acetylene, and purified acetylene upon green bananas. Exp. V.

and a photograph of the representative samples taken at the end of 120 hours is shown in fig. 4. The sections cut from the fruit were treated with iodine in potassium-iodide solution and show the disappearance of starch from the central portion of the fruit treated with acetylene. The check fruit and that treated with ammonia do not show this difference.

At the time these samples were taken, both those treated with acetylene and purified acetylene were yellow and well flavored, whereas both the control and the ammonia treated samples were mixed yellowish-green and greenish-yellow and were decidedly unripe. The starch test for the acetylene treatments was described as moderately high, while that for the check and ammonia treated was termed very high. The last two ammonia treatments were probably somewhat severe as the final ripening was not as good as that of the check and also, there were a few very small brown dots which were probably indications of injury at the points of entry. Furthermore, the divergence of the respiration curves for these two samples

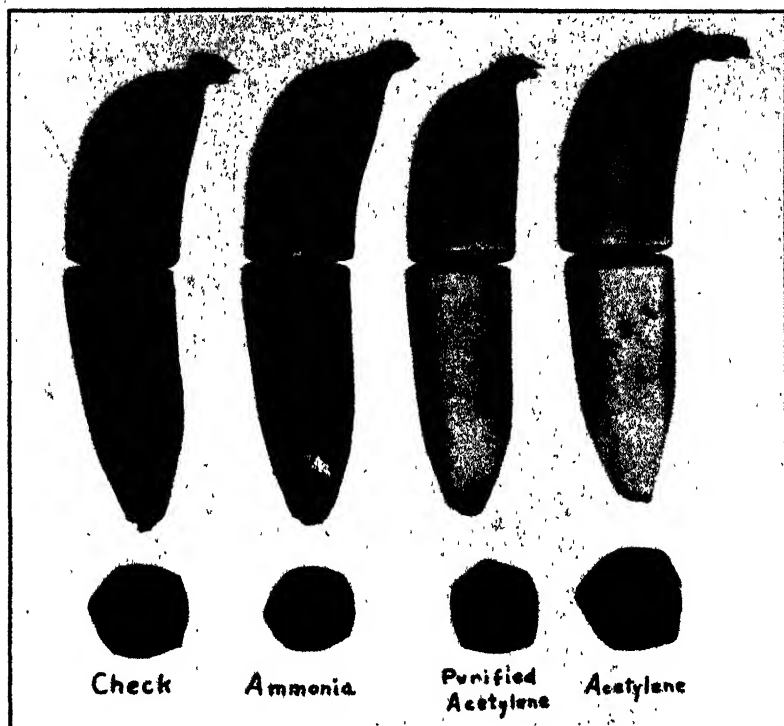


FIG. 4. Representative samples from the treatments in Exp. V showing color change and disappearance of starch with the acetylene treatments.

suggest that the ammonia treatment hindered ripening. From this experiment it appears that ammonia was not the active agent in hastening the ripening in the previous experiments, as certainly all traces of ammonia were removed in the purification in this experiment and the results obtained with the purified gas are practically the same as those with the carbide treatment. Undoubtedly, some of the acetylene was absorbed by the sulphuric acid used in the purification. There was no detectable odor in the chamber receiving the purified gas whereas the other chamber smelled strongly of acetylene. It seems established, therefore, that ammonia, as an impurity in the acetylene, is not responsible for the hastened ripening with the carbide treatment.

Acetylene from another source

DENNY (3), in his work on the coloration of lemons, did not obtain the same results with acetylene generated by the action of alcoholic potash and ethylene dibromide as with that from tanks. It seemed advisable, therefore,

to test this point in regard to bananas. Acetylene was generated from the above substances by a method described by SABENEJEFF (32) with the additional purification suggested by ZEISEL (40).

EXPERIMENT VI

In this experiment comparisons were made between the effects of purified acetylene, acetylene from ethylene dibromide, and ethylene. The procedure was similar to that of the preceding experiment except that the latter two gases were introduced into the air stream from bottles by displacement with water. The fruit used in this experiment had been in a ripening room with a kerosene stove for 24 hours before being obtained. All samples, including the control, ripened very quickly. The results appear in fig. 5, and the observations in table IV.

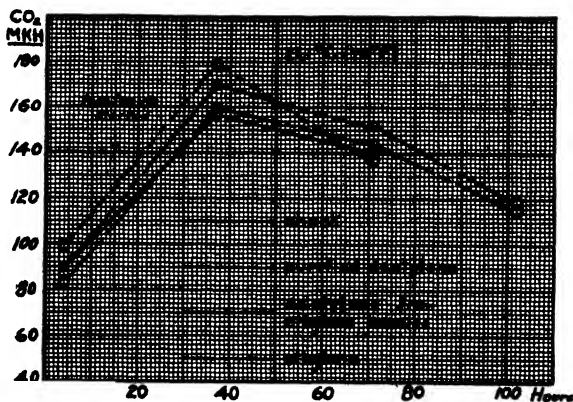


FIG. 5. Showing respiration of rapidly ripening bananas. Effect of gas treatments on respiration questionable. Exp. VI.

TABLE IV

OBSERVATIONS ACCOMPANYING RESULTS OF EXPERIMENT VI

TREATMENT	AT END OF 72 HRS.	AT END OF 103 HRS.	
	COLOR	COLOR	FLAVOR
Check	yellowish green (not uniform)	yellow and slightly greenish yellow, green tips	slightly astringent
Purified acetylene	pale yellow	yellow, two green tips	good
Acetylene from ethylene dibromide .	pale yellow	yellow, no green tips	good
Ethylene	greenish yellow, pale yellow	yellow, no green tips	good

It is quite clear from the graph that there was very little difference in the respiratory rate of the check and that of the sample treated with acetylene from ethylene dibromide. However, considering the differences in color and flavor, and the fact that the check ripened unusually fast, it seems that the lack of greater differences in the respiratory rate can safely be explained on the basis that the previous treatment had already accelerated the ripening processes. In order to check this point, this experiment was repeated.

EXPERIMENT VII

Halves of two hands composed a sample, each of which weighed about 1,700 grams. The check sample was from the same hands as the sample treated with acetylene from ethylene dibromide. The temperatures of the latter treatment were purposely kept slightly below that of the control to avoid any possibility of the temperatures being in its favor. Because of the importance of the temperatures in this case the actual data are presented in table V. It should also be stated, that the ethylene treatment

TABLE V
TEMPERATURE RECORDS FOR EXPERIMENT VII.

TIME	TREATMENT								
	CHECK			PURIFIED ACETYLENE			ACETYLENE FROM ETHYLENE DIBROMIDE		
	AIR	BATH	PULP	AIR	BATH	PULP	AIR	BATH	PULP
<i>hours</i>	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.
0.	21.1	21.1	20.	21.1	20.7	20.	21.1	20.4	20.
6.5	21.8	20.9		21.9	20.8		21.2	20.6	
8.5	21.9	21.1		22.0	21.1		21.4	20.5	
38.	21.7	20.9		22.0	21.1		21.2	20.8	
40.	22.0	21.1		22.6	21.1		21.5	20.6	
72.	22.0	20.9	22.2	22.5	21.4	22.2	21.4	21.1	
78.5	22.2	21.1		22.3	20.8		22.2	21.1	
80.5	22.0	21.1		22.2	20.9		22.2	21.1	
94.	21.9	20.8		21.1	19.4-21.1		21.8	20.4	
96.	21.9	21.1	22.2	21.9	21.1	21.4	21.7	20.4	21.4

in this case is not strictly comparable in that an electric light bulb was used as a heating unit whereas, in all other cases, immersion heaters were used. The effect light may have is unknown.

Unfortunately, the fruit used was not satisfactory as it was even older than that used in the preceding experiment. The results, fig. 6, indicate that the fruit was well started in the ripening processes at the beginning

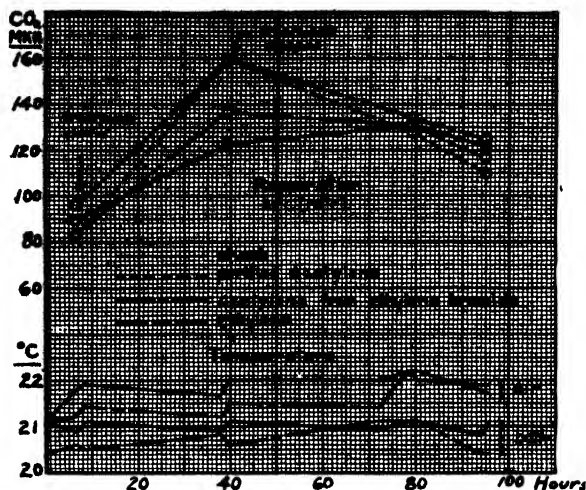


FIG. 6. Effect of purified acetylene, acetylene from ethylene dibromide and ethylene upon the respiration of ripening bananas. Exp. VII.

of the experiment. In spite of this, there was a slight but significant difference in the respiratory rate of the fruit treated with acetylene from ethylene dibromide as compared with that of the control. Also, at the end of the experiment, the check still had green tips while both of the acetylene treatments were uniformly yellow. The treated fruit was well flavored whereas the check was slightly green and astringent to the taste. In view of these differences, and in spite of the conditions it seems quite likely that acetylene, from either calcium carbide or ethylene dibromide and KOH will give similar hastening of the ripening of bananas. The effect of acetylene appears to be qualitatively similar to that of ethylene.

Discussion

A comparison of the respiration determinations of various samples is given in fig. 7. The curve, (number 3), which represents the average of several check samples, indicates, in general, the form of the respiration curve of normally ripening bananas held at 21.1° C. Sometimes the rise is a little more rapid, the peak higher and more pointed. On the other hand, when fruit has been subjected to conditions which affect it adversely, the curve may remain very flat for some time. This is illustrated by the curve of the chilled fruit, (number 1). Curve number 5 is that of the check in experiment VI, in which the treatments failed to show acceleration of respiration. The explanation for this lack of difference seems to be, that the fruit was already in the process of ripening very rapidly and near the peak of its activity. To substantiate this, is the fact that when

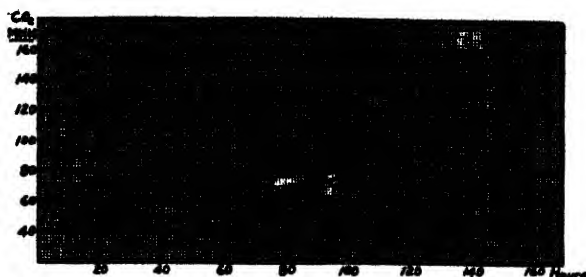


FIG. 7. Comparison of respiration of the checks of different experiments.

1. Check in chilling experiment, (check chilled).
2. Check of experiment in which acetylene treatment showed marked acceleration.
3. Typical ripening curve (average of several checks).
4. Check of experiment in which treatments showed only slight differences.
5. Check of experiment in which treatments showed differences which were questionable.

curve 5 is superimposed upon curve 3, (see dotted lines), the agreement seems to be very close. Curve 4 is that of the control in experiment VII, in which more difference was shown by the treatments. It will be seen that in this case also the fruit was ripening rapidly, but not as rapidly as in the other case, and that consequently, the differences due to the treatment were more evident.

Therefore, since the fruit used in experiments VI and VII was in the process of ripening very rapidly and near the peak of its activity, it is not surprising to find that the treatments did not cause greater acceleration.

The treatments appear to be most effective in shortening the initial period of low activity and consequently the greatest differences are found in the cases in which this period is of greatest duration, as in that of severely chilled fruit. It is also quite likely that once this acceleration of activity has commenced the presence of the gas is no longer necessary. This, however, needs experimental investigation. If the preceding hypothesis is correct, then such varieties as the Lacatan, which are ripened slowly and with difficulty, should respond similarly to the chilled fruit.



FIG. 8. Respiration of bananas as determined by various workers.

In fig. 8 are shown the determinations of various workers who have investigated the respiration of bananas. The curve of the average of several check lots is also repeated for comparison. From a study of these curves, it will be seen that these workers used bananas which were already well advanced in the ripening processes. This point was first established by E. F. HOPKINS.² As a matter of fact, LANGWORTHY (18) states that the fruit was "more mature than usual," also, that it hung in the laboratory over night; and GORE (9) says that the "bananas were on the point of turning yellow." OLNEY (25) makes mention of the relative maturity of the fruit used but even his "least mature" was far from green, as can be seen from the curve. Furthermore, his work being done in Chicago would make it even more difficult to obtain fruit which had not encountered favorable conditions for the ripening processes. By a comparison of these curves and also those in fig. 7, it seems safe to conclude, that in the case of the previously reported work, and in the experiments herein presented in which the controls ripened rapidly that the fruit was already well along in the ripening processes when the experiments were begun, and that furthermore, this was due to the fruit having previously been exposed to favorable ripening conditions or to substances such as ethylene, acetylene, or other volatile substances which would stimulate the ripening processes as indicated by a high initial respiratory rate and an immediate rapid rise of the same.

The work of HIBBARD (15) and WOLFE³ with ethylene would seem to fall in line with these observations and conclusions. The fruit which HIBBARD found to require 232 hours to color was probably chilled, and that "which had been exposed to low temperature for too long a period" was probably more than just chilled, or had also received other deleterious treatment. Low humidity sometimes has injurious effects which are similar to chilling, but the response of the fruit so exposed would not be the same. All the work herein reported was with very high humidity.

Though no previous work has been reported as to acetylene acting similarly to ethylene in the ripening of bananas, there is evidence in the literature which indicates that this is in line with its behavior. It has been reported as causing—nutations, (16, 17, 24, 29); changes in the chemical composition of germinating seeds and tubers, (10); decomposition of chlorophyll, (8, 11); production of intumescences, (35); breaking of rest periods, (23, 36, 37, 38); and inhibition of growth, (17, 39).

² HOPKINS, E. F. Respiration and ripening of bananas. Unpublished report to the United Fruit Co. 1-46. 1927.

³ WOLFE, H. S. The effect of ethylene on the ripening of bananas. Paper presented before the Amer. Soc. Plant Physiologists, Cleveland meetings, 1930.

Though the action of acetylene, ethylene, and propylene seems to be somewhat different from that of other substances, notably as regards the wide range of effective concentrations, there is still insufficient evidence to connect this behavior with the unsaturated condition of the molecule. Many other substances have been shown to be capable of hastening the ripening of fruits (34), and forcing plants (5, 6, 7, 33, 37). The action of these gases on horticultural material has been more completely presented in a paper by HARTSHORN.⁴

Summary

The experiments here reported show that the "carbide treatment" hastens the ripening processes of thoroughly green bananas as shown by the rates of softening, respiration, starch hydrolysis, flavor and color changes. That these effects are due to acetylene rather than ammonia or some other impurity in the gas seems fairly well established by the experiments in which the gas was purified. It has not been shown that no ethylene was present in the acetylene, but it seems very unlikely in view of the conditions under which the gas was produced and purified, and the low concentration of acetylene used in some experiments. There appeared to be no sharp limits to the concentrations of acetylene giving these results.

Though the difference in the respiratory rate may be considerably increased at a given time by the treatment, the maximum respiratory rate during the ripening may not be greatly changed by the treatment except in the case of severely chilled fruit. The effect appears to be mainly in the abbreviation of the period of low activity normally occurring at the beginning of the ripening processes or which may be considerably extended by unfavorable conditions such as chilling.

The condition of the fruit at the beginning of the experiment is of prime importance as to the results obtained. It is suggested, that the high initial respiratory rates determined by previous workers were due to the fact that the fruit was well advanced in the ripening processes, at the beginning of the experiments, due either to exposure to favorable ripening conditions, or to stimulation by ethylene, acetylene or other volatile substances of similar action.

The results with acetylene are in agreement with those obtained with ethylene, and also with its previously determined effects on horticultural material.

WASHINGTON, D. C.

⁴ HARTSHORN, ROBERT H. The effect of acetylene, ethylene and propylene on horticultural material. Cornell University Thesis, 1-38. 1929.

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WATER-SUPPLYING POWER OF THE SOIL UNDER DIFFERENT SPECIES OF GRASS AND WITH DIFFERENT RATES OF WATER APPLICATION¹

F. A. WELTON AND J. D. WILSON

(WITH FIVE FIGURES)

The water-supplying power of the soil has been investigated by a number of workers during the past 30 years. The soil-point method which is used in this study was described by LIVINGSTON and KOKETSU (3). This method has since been used to study the seasonal variations in the water-supplying power of the soil with reference to rainfall, evaporation rate and the growth condition of lawn grasses by LIVINGSTON and OHGA (4) and by WILSON (5). Other closely related investigations have been made by LIVINGSTON, HEMMI and WILSON (2) and by BALDWIN (1). An unpublished paper by WILSON and LIVINGSTON deals with the relations existing between the decreasing water-supplying power of the soil and the wilting and death of a number of grass species. Some of the observations made in the course of the last mentioned study suggested the experiments reported here.

During the past three seasons the senior author has been conducting an experiment on the use of water by various lawn grasses. This includes a series of seven plots, each of which is watered differently. Each plot is divided into three equal parts, one-third of which is planted to Kentucky blue grass, one-third to Chewing's fescue and the remainder to Washington bent grass. Water is added to five of these plots in measured quantities and at definite times and intervals. The other two plots serve as checks and receive only what water falls as rain. The grass is clipped and weighed at intervals, the length of these in days being determined by the rate of growth.

Only four of the seven plots were used in the study reported here. These included one check and three other plots which received 1.5, 2.0, and 3.0 times the normal, respectively. This "normal" is the average amount of rainfall for the months included in the growing season at Wooster, Ohio, as determined by the Weather Bureau station during the past 40 years. Thus the plot represented by 1.5 N received an amount of water which exceeded that which would normally fall on the plots by 50 per cent., the 2.0 N an excess of 100 per cent., and the 3.0 N exceeded the check by 200 per cent. Since the rainfall was very deficient during the summer of 1930 these relative values did not obtain, as will be shown later.

¹ Published with the approval of the Director of the Ohio Agricultural Experiment Station.

The methods used in making the water-supplying-power determinations were the same as those described by the junior author (5) in a seasonal study of lawn grasses in relation to the water-supplying power of the soil. The usual form of porous porcelain soil point was employed. This instrument has an absorbing surface of approximately 12 square centimeters. The points were placed with the aid of a dibble. The depth of insertion was such that the mean depth of the absorbing surface was six centimeters. The period of exposure was one hour. Four soil points were used on each grass species on each plot for each set of determinations. Thus, the values representing the water-supplying power of the soil in each instance are the average of four instruments. The determinations were made at irregular intervals during the months of June, July and August, the dates being determined largely by the general weather conditions.

The state of Ohio experienced a very severe drouth during the season of 1930 and the months of June, July, and August were especially dry, warm and sunny. As a result not only the check plot mentioned above, but many lawns suffered severely from a lack of soil moisture. The fact that the grasses on the check plot became very brown and were retarded in growth while those on the artificially watered plots remained green and grew well made this an excellent season to carry on this water-supplying-power study. The rainfall for May at Wooster, the data of which are included here because of the influence of the precipitation of this month on the soil moisture conditions in early June, was only 40.5 per cent. of the normal. For the months of June, July and August the percentages were 71.9, 42.1, and 74.8 of the normal, respectively, or the rainfall for the four months taken as a group was 56.8 per cent. of the normal. In comparison with these data, the evaporation totals, as determined with blackened, standardized LIVINGSTON spherical atmometers, were very high. For the months of May, June, July and August the rates were 19.4, 66.6, 77.1 and 48.0 per cent., respectively, in excess of the average for the same months of 1928 and 1929. If the four months are considered as a single period, the excess is 52.8 per cent. over the 1928-29 average. The mean temperatures for these four months were from one to three degrees F. above their normals. This combination of low rainfall and high evaporation rates, together with an excess of sunshine and temperature, brought about the very severe drouth experienced at Wooster in 1930.

Since the rainfall for the period including the months of June, July and August was only about 60 per cent. of the normal, the check plot did not receive its usual amount of water. This means that the artificially watered plots instead of receiving the indicated percentages in excess of the check actually received more than these. For instance the 1.5 N plot received 83 per cent. more water than the check instead of the indicated 50 per cent.

Thus 1.5 N became 1.83 N while 2.0 N was 2.67 N and 3.0 N was 4.33 N when these values are computed on the basis of the check. This variation in the relative amounts of water applied to the different plots cannot be avoided since in years when the rainfall exceeds the normal there is no practical way of decreasing the amount falling on the plots.

The water-supplying power of the soil in a restricted area having a uniform slope and vegetation covering is chiefly influenced by the quantity of water falling as rain and by the evaporation rate. If water is added artificially it too must, of course, be considered. Figure 1 shows some of the



FIG. 1. Graphical representation of rainfall (heavy, vertical lines), evaporation rates (dash line), and the water-supplying power of the soil of the check plot (full line closely paralleling the base) and an average of the values for the plots receiving 1.5 and 2.0 times the normal falling as rain (upper full line). The capital letters at the top indicate the dates of adding water to the 1.5 and 2.0 N plots.

relations which existed over a period of three months between the water-supplying power of the soil in the grass plots studied and the environmental factors of rainfall, rainfall plus irrigation, and the evaporating power of the air. The full line, which closely follows the base line of the graph over part of its length, represents the water-supplying power of the soil on the check plot. This plot, as stated earlier, received only what water fell as rain during the period of observation. The values indicated are averages of those obtained for all three of the grass species on the plot. The full line in the upper part of the graph shows similar values obtained by averaging those obtained on the 1.5 N and 2.0 N plots. The dash line indicates the average daily evaporation values, in terms of cubic centimeters, the aver-

ages being determined for intervals varying in length according to the dates on which soil-point determinations were made. The heavy vertical lines represent the rainfall in inches and indicate the dates on which the rains occurred. The dates on which water was artificially added to the two plots included in data shown in the upper full line are indicated by the letters at the top of the graph.

The water-supplying power of the check plot was very low in early June after the very light rainfall of May. The rains of June 16, 17 and 18 moistened the surface soil enough to give a value of 424 on June 19. This was not equaled again until August 26, when 446 milligrams of water were absorbed per soil point per hour at a depth of six centimeters. On July 3 the water-supplying power on the check plot had fallen to a value of 32 in spite of the rains of June 24, 26 and 30. The light rains occurring between July 3 and August 14 had very little influence on the moisture content of the soil since none of the soil point values exceeded 31 milligrams during this period. The grasses on this plot remained very brown and in a poor vegetative condition throughout the period. The fescue was least injured, the other two species suffered equally as far as could be determined by a casual examination. The water-supplying power of the 1.5 N and 2.0 N plots showed maximum values on the same dates as the check, indicating that these were dates of high moisture values even on well watered areas. The low rainfall and high evaporation rates of the last half of July brought about a gradual drying out of the soil even on these artificially watered plots. The very low value of July 31 was unexpected but it may be noted on the chart that this came after a period of high evaporation and low rainfall and, what is also significant, eight days after the last previous application of water. The total evaporation from the blackened sphere for this period of eight days was 512 cc. or sufficient according to WILSON (5) to correspond to a very considerable decrease in the water-supplying power if no rain intervened. Only about 0.35 of an inch fell during this period. Each of the three species of grass remained in good condition on these two plots throughout the summer. The water-supplying power of the 1.5 N plot, which was of course drier than the 2.0 N, showed no average values for the three species which were less than 500 milligrams and only two of the 33 determinations made on this plot were below this amount. These were both on Kentucky blue grass. This value of 500 mg. per soil point per hour at the 6-cm. depth was the one determined by WILSON (5) to represent a supplying power which should always be sufficient for good growth of lawn grasses. That this was also sufficient in this instance is shown by some of the data presented in the following paragraphs.

Figure 2 presents a comparison between the water-supplying power of the soil for three of the plots and the yield of grass which they produced

during the period made up of the months of June, July and August. The water-supplying-power values are represented by the dash line. The value shown for each plot, or water treatment, was arrived at by including all three species for the eleven dates, that is, an average of 33 determinations. The yield values, shown by the full line, include two species only—blue grass and fescue—and represent the total weight in kilograms of grass removed from each plot during the three months mentioned above. The

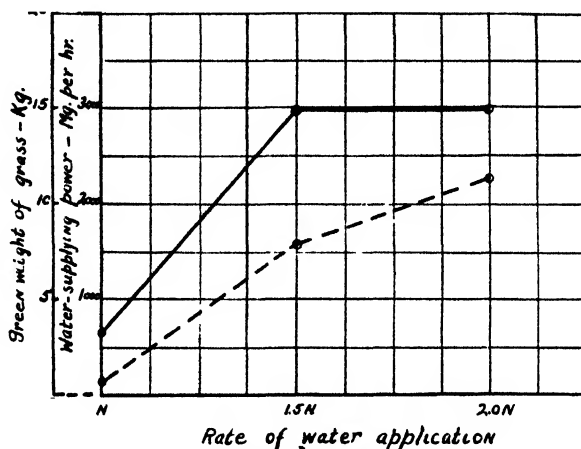


FIG. 2. The water supplying power of the soil of the check plot and of those receiving 1.5 and 2.0 times the normal falling as rain (dash line) compared to the yields of grass for the months of June, July and August, 1930.

yield of the 1.0 N plot was low in comparison with that on the other two plots. On the other hand, the yield of the 1.5 N plot was nearly equal to that of the 2.0 N area. The average water-supplying-power value of 1.0 N was only 98 milligrams and if the two determinations of June 19 and August 26 are not included the average is only 23 milligrams for nine different dates. This value is far below the 500-mg. value previously mentioned and is also well below the value of 100 mg. which LIVINGSTON and OHGA (4) and WILSON (5) found to be a minimum below which grasses would be definitely injured through a lack of water. The average water-supplying power on the 1.5 N plot was about 1600 mg. or well above the 500-mg. value. The corresponding average on the 2.0 N plot was about 2,250 mg. Thus while an average water-supplying power below 100 mg. was insufficient for good growth of grass, one of 1,600 mg. was sufficient and nearly as good as one of 2,250 mg. The applications of water beyond that placed on the 1.5 N plot seems to have been unnecessary even in a season as dry as that of 1930. Also it is possible that the use of some amount even less than 1.5 N would have given nearly as good growth.

Figure 3 is similar to figure 2 except that it includes data from the 3.0 N plot and covers only the months of June and July, during which eight determinations of the water-supplying power of the soil were made. The slope of both lines is again much greater from 1.0 N to 1.5 N than from

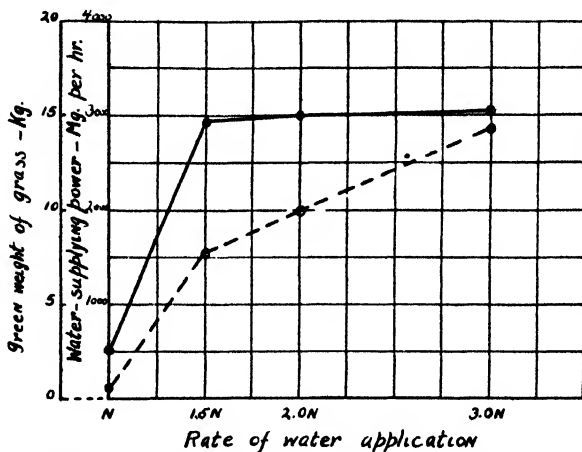


FIG. 3. Similar to figure 2. Data for the 3.0 N plot is added but only the months of June and July are represented.

1.5 N to 2.0 N and the increase in yield was almost negligible from 2.0 N to 3.0 N. The water-supplying power of the soil continued to increase up to 3.0 N where it reached a value of over 2,800 mg., but this was clearly of little importance in increasing the yield of grass over that on the 1.5 N plot with a water-supplying-power value of about 1,500 mg. If the use of water in excess of that added to the 1.5 N plot was questionable in considering the data of figure 2, then the addition of the extra amount on the 3.0 N plot as shown in figure 3, was clearly unnecessary.

The water-supplying power of the soil under each of three grasses at three different water treatments is shown in figure 4. Each value indicated is the average of the eleven determinations made on a given plot section during the months of June and July and August. The full line shows the water-supplying power of the sections bearing Chewing's fescue. The dash line represents the Washington bent grass and the dotted line that of the portions planted to Kentucky blue grass. During these two months the soil under the Kentucky blue grass supplied less water to a soil point than that under either of the other two species. However, it was not very different from the sections bearing Washington bent grass, being equal to or greater than the latter in some instances. The average values obtained for Chewing's fescue were considerably above those for the other two grasses, being about 60 per cent. greater than that for Kentucky blue grass

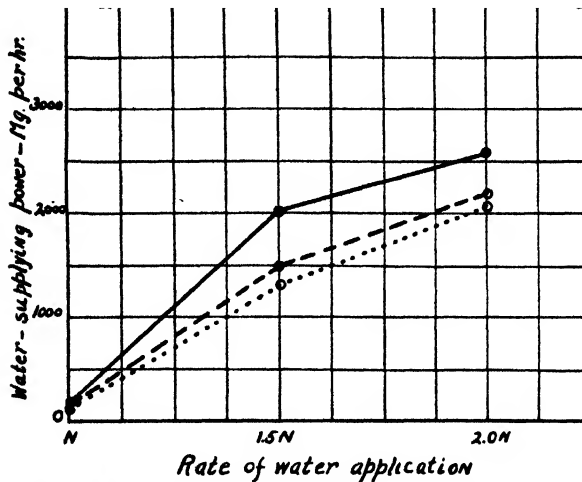


FIG. 4. A comparison of the water-supplying power of the soil under Chewung's fescue (full line), Washington bent grass (dash line), and Kentucky blue grass (dotted line), with three water treatments for the months of June, July and August.

on the 1.5 N plot and on some of the dates involved it was two or three times as great. Since equal quantities of water were applied to each species of grass on each plot this difference in the water-supplying power of the soil with the different grasses must have been due to some difference in the rate of water use. The narrow-leaved fescue apparently did not place as great a demand on the available soil moisture supply as the other two forms and this may be one of the reasons why the fescue remained green much longer than the bent or blue grass with the beginning of drouth conditions during the past summer. In an unpublished study by the junior author, in which the response of 17 different species of grass to a progressive decrease in the water-supplying power of the soil was observed, it was found that the fescues as a group were more drouth resistant than the bent or blue grass forms. This study also indicated that none of the forms tested, with the possible exception of some of the fescues, could survive an extended period during which the water-supplying power of the soil was below 50 mg. This was the case during the past summer for the three species growing on the check plots. The water-supplying power of the soil under all three species was below this value during most of the summer, the value of the fescue section being nearly as low as that for the other two sections as is shown in figure 4.

Figure 5 is similar to figure 4 except that it includes the data for the 3.0 N plot and covers only the months of June and July during which eight determinations of the water-supplying power of the soil were made. The

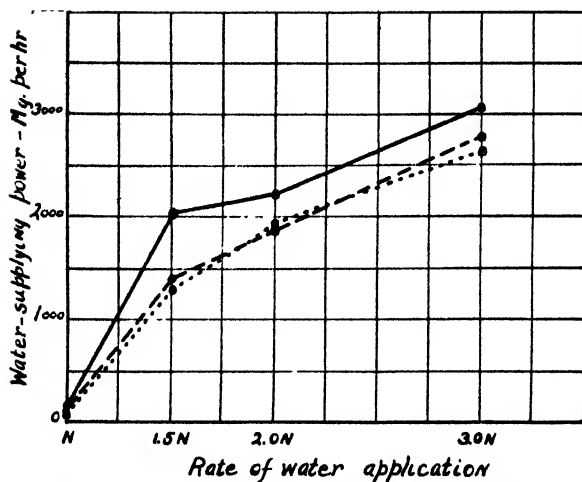


FIG. 5. Similar to figure 4. Data for four water treatments for the months of June and July only.

data are similar to those shown in figure 4. The values for the fescue are still well above those for the other two species, being relatively less in excess at 2.0 N and 3.0 N than at 1.5 N. In this case the blue grass section of the 2.0 N plot gave a higher average value than the bent grass section, but indicating again that there was little difference in the rates at which these two species use water.

Summary

The water-supplying power of the soil under three different species of lawn grasses was determined at intervals in the months of June, July and August during the summer of 1930 at Wooster, Ohio. The grasses were Kentucky blue, Chewing's fescue and Washington bent. Four different water treatments were included in the study. One plot received no water in addition to that falling as rain, a second received an additional amount equal to 50 per cent. of the normal rainfall, a third 100 per cent. of this normal and a fourth 200 per cent.

The usual form of porous porcelain soil point was used, this being inserted to a depth of six centimeters and left in the soil for a period of one hour.

Data on such environmental factors as rainfall and evaporation rates are given. The rainfall was very low, only about 60 per cent. of the normal for the months of May, June, July and August. This deficiency, of course, disturbed the intended ratio of 1 to 1.5, to 2.0, and to 3.0 for the different plots. The evaporation rates were far above the average for the summers

of 1928 and 1929. This high water loss aggravated the dry conditions which normally would have resulted from such a lack of rainfall as existed and in consequence a very severe drouth was experienced.

The water-supplying power of the soil on the three sections of the check plot was below the critical value of 100 mg. during most of the 3-month period involved and the grasses were brown and dry. The other plots were usually well over the value of 500 mg. above which most lawn grasses do not suffer from a lack of soil moisture, and each of the three species remained in good condition throughout the experimental period.

The yields were nearly as good from the 1.5 N plot as from either of the others, each of which received an additional amount of water, indicating that amounts in excess of those which will maintain a water-supplying power of the soil at 500 mg. or above are not required to obtain good top growth of lawn grasses.

The water-supplying power of the soil under the fescue was greater in nearly every one of the 41 determinations involved. Its average value for 11 dates on the 1.5 N plot was about 60 per cent. greater than that for the Kentucky blue grass. The values for the latter and Washington bent were quite similar, the bent usually being slightly higher. These results suggest that the narrow-leaved fescue places a smaller demand on the available soil moisture than the other two broader-leaved forms. This, together with the fact that it enters a drouth period with a greater reserve of soil moisture is probably a significant factor in enabling this species to survive drouth periods better than many other forms such as the bents and blue grasses.

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MOVEMENT OF WATER IN PLANTS AS AFFECTED BY A MUTUAL RELATION BETWEEN THE HYDROSTATIC AND PNEUMATIC SYSTEMS¹

E. M. HARVEY

(WITH FIVE FIGURES)

When one considers the vast accumulation of literature dealing with the problem of water movement in plants, the paths of its conduction, the nature of the structural elements involved, the possible sources of energy required, and the mechanics of its application, it seems rather late to offer another suggestion. Yet the purpose of this paper is to call attention to a certain phenomenon of water movement in plants, and to suggest an explanation of it.

Recently during a series of studies on the paths of conduction, or rather, of least resistance, between different parts of apple branches by means of dye solutions, the following situation was repeatedly observed. Whenever suction is applied to one cut-off branch and a dye solution to another, even though the latter is located at considerable distance from the former, the dye enters the main stem and then streams, not only through the conducting elements toward the source of suction pull, but also rapidly and directly away from it. There were certain variations in the path taken by the dye solutions, depending on whether or not the suction was applied "above" or "below" the source of the dye solution, or on the relative ages of the two branches under observation. These details will be considered later, but the interesting thing always evident was this, that there seemed to be some force acting definitely and in somewhat the same strength, both *toward* and *away from* the source of suction.

This opposite-acting force was at first assumed to be saturation deficit, but the idea was discarded when it was found to require hours for dye solutions without suction to traverse distances which could be accomplished in 2 to 15 minutes with suction.

It was then thought possible, since the suction connections had always covered the bark of the cut-off branches, that the negative pneumatic tension had been propagated more rapidly through the cortex than through the xylem to points *beyond* the branch receiving the dye solution, and that the negative tension at such points had in some manner been translated radially to the xylem, thereby causing the dye solution to be drawn away from the suction force. Again, this idea had to be abandoned when it was found that if the bark were completely removed, the same phenomenon persisted.

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It now became necessary to face the conception of a hydrostatic-pneumatic system in the xylem, a conception which has been so well brought forward through the extensive and critical researches of MACDOUGAL (1, 2) and recently and, more particularly, by MACDOUGAL, OVERTON and SMITH (3). In the latter work it was shown that there is not only a definite water conducting portion in each annual ring, but also a gas-containing one. These portions of an annual ring were designated, early spring, spring and late summer wood, and the function of each as regards water conduction or gas-containing seemed to be more or less fixed for each species. This work, therefore, presents definite information as to the relative location of the hydrostatic and pneumatic systems of certain plants. OVERTON (4) has just announced that the relative areas occupied by these two systems in a given plant vary with the season.

The results of a particular series of experiments of MACDOUGAL, OVERTON and SMITH (3) bear directly upon the present question. These investigators fixed several air-connected manometers into a tree at varying distances apart and then applied suction. The manometers located directly above and below the point of suction responded quickly to the negative tension, but those on the opposite side of the tree trunk responded very slowly or not at all. The authors, however, observed no consistent response in the hydrostatic system to variations of tension in the pneumatic.

But in order to explain the results to be reported in the present paper, it seems necessary to assume a definite interaction between the hydrostatic and pneumatic systems, and that the pneumatic system can, under certain conditions, become a factor in aiding water movement in plants.

To illustrate this supposed interaction between the two systems, a few typical experiments will be described.

I. The quickest and simplest type of experiments for showing an interaction between hydrostatic and pneumatic units² in plants is as follows:

An apple branch,³ say two to four years old, is connected with a source of suction at the basal end. Now cut off a small side-branch leaving a stub at least 4-6 cm. in length. Insert this stub into a beaker of dye

² By the term hydrostatic or pneumatic "unit" is meant that definite portion of xylem tissue which is directly connected with, and in the main normally supplies, any given side-branch, so that such a "unit" may be either relatively large or small depending upon the size of the branch under consideration.

³ All branches or parts of branches used were freshly cut, within a few minutes before each experiment, from much larger branches which had been brought from the orchard, usually within 24 hours.

Such large branches were left out-of-doors where conditions were as in the orchard, that is to say, moist, and temperature low, but not below 0° C. All material was in the state of winter dormancy.

solution and start the suction. Sometimes within a few seconds, usually within five minutes, the dye will have been drawn to the main stem, and moved from the point of juncture, "downward" toward the source of suction, but at the same time a part of the stream of dye solution will have moved approximately an equal distance "upward"; that is to say, directly away from the source of suction. This experiment may be performed with or without removal of the bark. However, if the bark is previously removed, the movement of the dye can be followed readily; but if the bark is left intact during the experiment, it should be removed afterwards in order to observe results more easily. Fig. 1 shows the results of two such tests. Branch A was 4 years old and B was 3. The side branches (b-b) to which

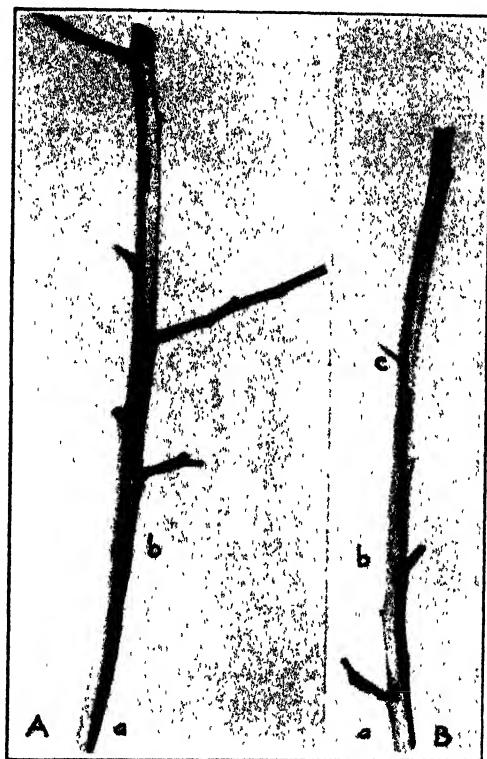


FIG. 1. Showing the movement of dye solutions in small branches when suction was applied at *a* and the solution at *b*.

the dye solutions were connected were each one year old. The duration of the experiment was 5 minutes and the negative tension was equivalent to nearly 700 mm. of Hg. Under these conditions the dye solution was drawn entirely to the base (*a-a*) of each, or 14 cm. In A the dye solution also

streamed along a line directly upward from *b* to a distance of 27 cm. In B, there was a corresponding upward streaming of the dye from *b*, but it reached a point only 17 cm. above *b* on account of having been largely deflected by branch *c*, one side of which the stream of dye entered and moved upward for about 10 cm. In such experiments as these the dye never leaves the hydrostatic unit of the side branch through which it is introduced. But if there is another side branch having a portion of its hydrostatic unit in common with the first, then the dye will freely enter the other through, and only through, the common unit and will remain within it.

This behavior of dye solutions in small apple branches is also characteristic for many other plants. The same behavior was noted for the Italian prune, pear, sweet cherry, sour cherry, *Amelanchier alnifolia*, *Crataegus douglasii*, *Rosa* 2 spp., red raspberry (var. Cuthbert), Loganberry, Evergreen blackberry (*R. laciniatus*), *Juglans cinerea*, *J. regia*, *Corylus avellana*, *Castanea dentata*, *Quercus garryana*, *Acer macrophyllum*, and *Salix* spp. Only two species tried have failed to respond in a manner as described above. These were *Fraxinus oregana* and the black raspberry (var. Plum Farmer).

II. A second type of experiment was tried where the procedure was exactly the reverse of the preceding, that is, the suction was applied to a side branch and the cut-off base was placed in the dye solution. The results, however, were the same as before, for when branches, which had been treated by this method were compared with specimens such as shown in fig. 1, it was difficult to distinguish them. The dye was drawn up from the base through the hydrostatic unit of the side branch in question, a portion of the dye passed into the branch, but the rest streamed up the stem beyond the side branch apparently against the suction force.

III. Experiments of type II above were modified to the extent that instead of placing the basal end into the dye solution, the stem was cut off above the suction attachment and that end placed in the solution. The resulting picture was somewhat different, although it still gave evidence of an interaction between the hydrostatic and pneumatic units. The characteristic results of this procedure are shown in B of fig. 2. Here the suction was applied at *b*, with the dye solution entering at *a*. The dye moved freely toward *b* in a rather wide stream, the middle part of which entered branch *b*, but mostly through its adaxial side. The outside portions of the stream passed around the base of *b* and moved rapidly in two parallel bands down the main branch for 24 cm. The right hand band (see fig. 3 B) was itself divided by the presence of another side branch at *c*. The dye did not enter that part of the hydrostatic unit which supplied the outside face of *b*. The situation is relative, however, for if suction is prolonged, the dye will cross over into the remaining part of *b* fairly completely. The duration of this particular experiment was 6 minutes only.



FIG. 2. A. The direct and indirect effects of suction at *a* with dye solutions admitted at *b* and *c*. B. The effects when suction was applied at *b* and the dye solution at *a*.

IV. While the foregoing experiments seem to indicate an interaction within a single hydrostatic-pneumatic unit, and that this interaction causes definite water movement, they have not furnished evidence as to whether or not there is an interaction between separate hydrostatic-pneumatic units. It has been assumed so far, that the water movements recorded, especially those away from the source of energy, were caused by a more rapid propagation of negative tension through the pneumatic units, so that this tension quickly passed around the greater resistance in the hydrostatic unit. In such situation it was then capable of applying itself to all points along the latter. Whenever the tension was transmitted in this manner to somewhat distant points, it caused water movements which appeared paradoxical.

In all experiments described, the dye solution and suction force were applied to the same hydrostatic-pneumatic unit. But the following experiments were designed to yield information concerning a possible interaction between relatively distant hydrostatic-pneumatic units.

The results of a typical experiment of this sort are shown in fig. 3. The material used was an apple branch, the main stem of which was 7 years old, and, near the bottom of the photograph, 5.8 cm. in diameter. Suction

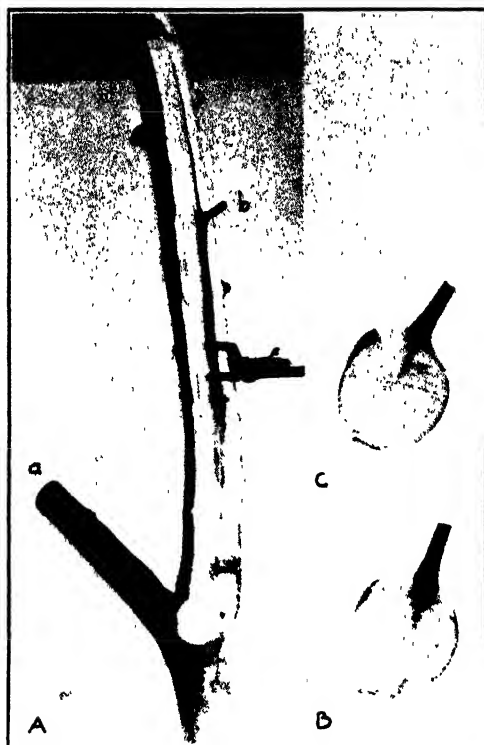


FIG. 3. A. Showing the mutual relation of the hydrostatic-pneumatic unit of branch *a* to that of branch *b*. B and C' are cross-sections just below and above *b* of A.

was applied at branch *a*. A dye solution was connected to the small side branch *b* which was situated 135° around the stem from branch *a*, and about 35 cm. above it. Suction was allowed to act for 40 minutes. The bark was then removed as shown. It was observed that the dye solution had entered the main branch and traveled 19 cm. upwards and about 40 cm. downwards. It is here admitted that there are forces, *e.g.*, saturation deficits, within such a branch capable of causing the dye solutions to be drawn in without suction, and afterwards the dye may be distributed in practically the same manner as in all cases described. There is this difference, however, namely: with suction the distribution of the dye may take place in a few minutes while without it, many hours are usually required to produce a corresponding effect.

In the results of this type of experiment, there are some points of especial interest. First, it was apparent that there was a cross transference of negative tension between separate pneumatic units, but practically no cross-transference between corresponding hydrostatic units. Note C

and B in fig. 3. These are photographs of cross-sections made just above and below *b* to show how strictly the dye solution is confined to the hydrostatic unit of *b*. At no point below did it show any tendency to leave this unit in order to move toward the source of suction at *a*. This result is interesting because it indicates that the pneumatic units of a stem, being relatively more in communication than the corresponding hydrostatic units, can transmit a negative tension from one hydrostatic-pneumatic unit to another, and as a consequence cause work to be performed in other, perhaps distant, hydrostatic units.

Experiments of the above type were carried out with a number of different combinations of "hook-ups," some of which yielded surprising results. An interesting, though simple one, is shown at A in fig. 2. The main stem was 7 years old and about 5 cm. in diameter. Branch *b* was 6 years old, branch *a*, 5 years and the stub *c* 2 years old. (The entire branch was cut back after the experiment while observing results). Suction was applied at *a*; the entire cut surface of *b* was placed in a solution of light green dye; and *c* was connected with a solution of trypan blue. After suction had acted for 35 minutes, the experiment was stopped and the bark removed. It was found that the light green dye had entered at *b* and passed over into *a*, but only in the hydrostatic unit which branches *a* and *b* shared in common. However, in addition, the green dye had streamed down the main stem for 44 cm. from the junction of the two branches. The cross-section of the main stem (now at the bottom of the photograph) showed the green color as a semi-circular band outlining the hydrostatic unit supplying the adaxial faces of *b* and *a*. The trypan blue entered at *c*, but since this shoot was a relatively recent addition to this branch system, it possessed only a thin hydrostatic-pneumatic unit which had little or no hydrostatic connection with *a* or *b*. As a result the dye did not enter branch *a* but streamed down the main stem in its own tortuous (at the beginning) and shallow hydrostatic unit for 35 cm. running directly away from the source of negative tension.

Another experiment of the same general type as the above will be described. The general situation at the end of the experiment is shown as fig. 4. It was carried out with an apple branch 8 years old and over three meters in length. Suction was applied at *a*, a branch 5 years old. Five mercury manometers, air-connected, to record pneumatic tension, were fixed in the main stem as follows: No. 1, directly below *a* at 27 cm; no. 2, also directly below at 89 cm; no. 3, directly above *a* at 21 cm; no. 4, also directly above at 73 cm; and no. 5, directly opposite from *a* and at a distance of 110 cm. Then an eosin solution was connected to the three year old branch *b*, situated about 90° around the trunk from *a*; trypan blue solutions were attached to the two-year old branches *c* and *d* situated

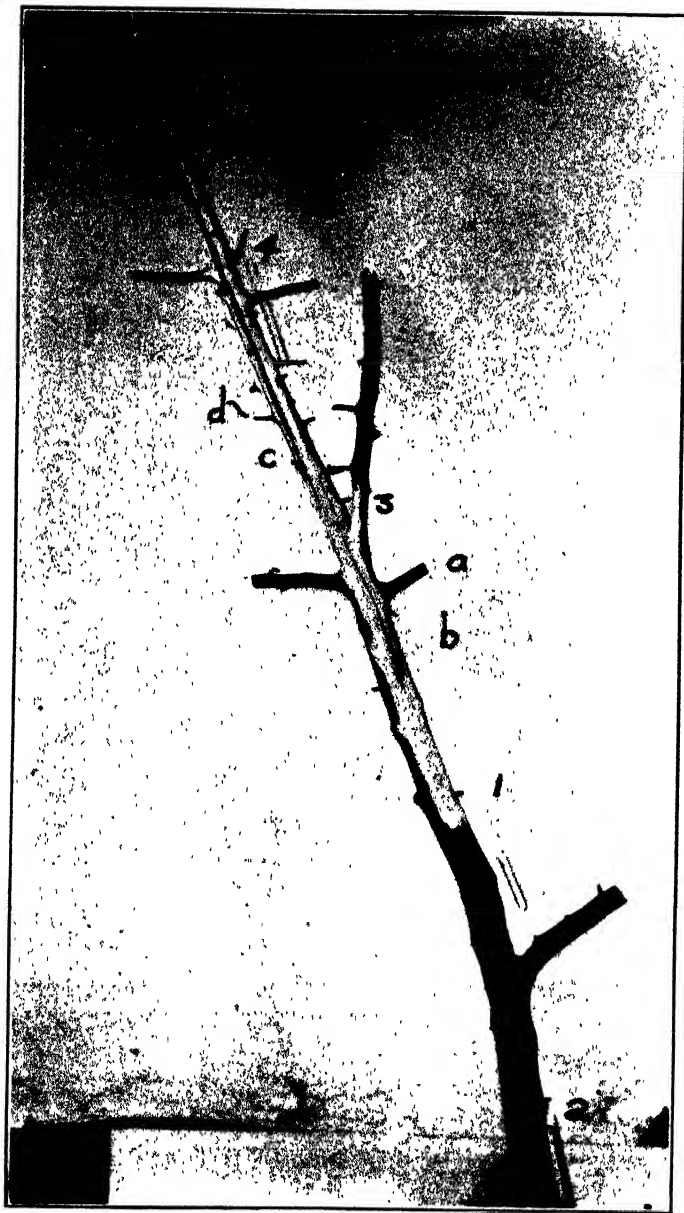


FIG. 4. Showing the arrangement and final result of the last specimen described under section IV.

at 130° and 143° around the trunk respectively; and finally, light green solution was attached to the two-year old branch *e*, situated approximately at 180° around from *a*.

Suction was started and in less than 10 seconds the Hg in manometer no. 3 was drawn into the boring, the negative tension having had to exceed 18 cm. of Hg to produce this effect. Manometer no. 2 quickly registered 14 cm. and no. 1, after a few minutes showed a negative tension of 8 mm. At the same time, no. 4 showed a tension of 12 mm. One-half hour later no. 5 indicated a trace of negative tension, possibly 2 mm. In the meantime the entrance of the dye solutions was being observed. This was done by stripping off bark above and below connection points. It was noted especially, how, in spite of the fact that manometer no. 5 had not yet recorded any negative tension, the dye solution had entered at *e*, located only 17 cm. below this latter manometer, and had moved both upward and downward 3.5 cm. and 4.5 cm. respectively in 15 minutes. The experiment was stopped in one hour and thirty minutes. The extent of the movement of the dye solution was as follows: From *b* the eosin moved upward 37 cm. and downward 18 cm.; from *c* the trypan blue moved upward 85 cm. and downward 12 cm.; from *d* the same dye moved upward 48 cm. and downward 16 cm.; and from *e* the light green had gone upward 32 cm. (but had been almost completely blocked by the boring for manometer no. 5). and downward 68 cm.

The results of this experiment indicate how water movements can be induced in a number of hydrostatic-pneumatic units simultaneously, by a negative tension originating in one of them, and being transmitted to the others through the several pneumatic units.

The responses of the pneumatic manometers in this experiment agree, in the main, with the more extensive results recorded by MACDOUGAL, OVERTON and SMITH (3). But it is interesting that water movement is initiated in a hydrostatic unit in response to a negative-tension originating in a relatively distant one, even though that tension is not strongly indicated by means of a pneumatically connected manometer in the vicinity of the former.

It should be mentioned in passing, that the relative location of the hydrostatic and pneumatic systems in each annual ring of the apple tree, during December is this: the hydrostatic system occupies the "early spring" and "spring" woods, and the pneumatic system the "late summer" wood only. Whether or not the pneumatic system encroaches upon the other during the summer months has not been determined, but considering OVERTON's (4) findings, it would seem likely.

V. A model was constructed to illustrate the possible mechanism by which a hydrostatic and a pneumatic unit in the plant interact. A model

of the sort is shown in fig. 5. It is extremely simple and can be assembled in any laboratory within a few minutes. The glass tubing used has an outside diameter of about 7 mm. That portion of the model indicated by cross-hatching is tightly filled with white sand of 20-40 mesh, and kept in place by cotton plugs. Tube A is connected to an aspirator for suction,

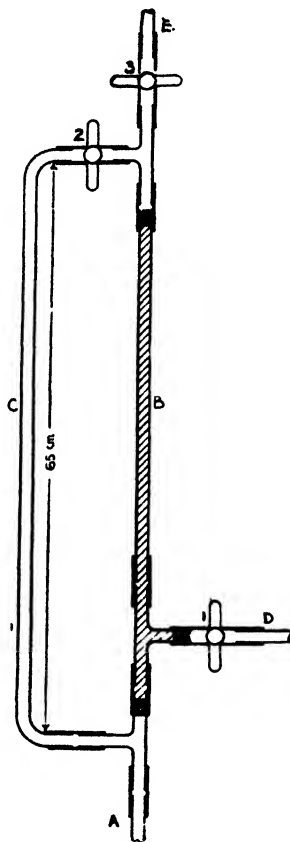


FIG. 5. A mechanical "hydrostatic-pneumatic unit."

D is connected to a short glass tube having a right angle bend so that it can be transferred at will from a beaker of distilled water to one of dye solution or *vice versa*, and E is connected with a distilled water supply. To make the demonstrations, close clamp no. 2, open clamps nos. 1 and 3. After E and D are connected to their water supplies, start aspirator. When section B has thoroughly filled with water, it represents an hydrostatic unit of a branch and a cut-off side branch, through which a dye solution is to be drawn. C of course represents the corresponding pneumatic unit, but at the first stage of the demonstration it is prohibited from act-

ing by the closure of clamp no. 2. Now with aspirator still on, close clamp no. 3 and place D in a dye solution. When clamp no. 3 is reopened the dye solution will now enter into the "main branch," and will, if the negative pressure is kept constant, stream toward A only. Next open clamp no. 2, thus bringing the pneumatic unit into action. At once the dye solution will commence to stream "upward" through B also and will continue until it reaches the end of the tube.⁴ The behavior of the dye solution in the model resembles so much what one sees while performing the type of experiment recorded in section I, that it is difficult to refrain from judging the two mechanisms essentially the same. However, within a plant, the negative tension would be transferred from the pneumatic to the hydrostatic unit at numerous points, if not almost continuously, along the path. It may be also suggested that if a number of such models, or mechanical hydrostatic-pneumatic units, should be connected through their "pneumatic systems," each one would perform the same work when a negative tension is applied to any one of them. The interaction of this multiple system might be compared to what goes on in the several hydrostatic-pneumatic units of a stem. Yet the comparison is not entirely fair, on account of the obviously greater resistance between pneumatic units in a stem, and the loss of tension from air entering through lenticels.

In this paper there has been enough "discussion" in the introductory paragraphs and during the description of experiments, to leave little more desirable. It remains to say, however, that the significance, if any, of the results reported herewith, lies in the possibility of a functional relation between the hydrostatic and pneumatic systems in plants affecting water movements. How important the function really is under natural conditions, while transpiration is active, this paper offers no evidence. Nevertheless, the seeming fact that there is a mechanism which permits a negative tension in one hydrostatic-pneumatic unit of a stem to be propagated relatively quickly to other units, and in them to cause work to be done (*i.e.*, movement of water), is worthy of consideration. Whenever calculations are made as to the total energy required to move a given quantity of water through a given length of stem in a given time, the recognized high hydrostatic resistance of the xylem is always taken into account. The resulting figures are frequently very large. Is it not possible that a more facile transmission of energy through the pneumatic units, and the consequent application of this energy all along the hydrostatic path, might lower the value of the previously calculated total energy requirements for the rise of the transpiration stream?

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⁴ To clean apparatus close clamp no. 2, open clamp no. 3, and transfer D back to the water supply, leaving suction on.

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EXTERNAL POLARITY POTENTIALS IN THE APEX OF THE DOUGLAS FIR BEFORE AND AFTER MECHANICAL STIMULATION

E. J. LUND

(WITH FOUR FIGURES)

Introduction

In previous work on the electric polarity in the Douglas fir LUND (1, 2, 3) attention was limited to the phenomena occurring in the main axis and lateral branches of the tree. The measurements of P. D. which incidentally were made upon the apex of the main stem and the first apical whorl of lateral branches showed a small or at times even a reversed polarity LUND (2, tables 1 and 2). Since most of these measurements were necessarily made out of doors under conditions which involved mechanical stimulation of the tips by slight wind movements, and also involved a method of taking readings of the P. D.'s by gently bending the tips of the apex and branches into cups, it seemed desirable to make a separate study of the electric polarity of the extreme apical region of the main axis and lateral branches under more uniform laboratory conditions.

The present measurements were made upon actively growing freshly cut tips whose cut ends were immersed in water. Electrode contacts were made as usual with narrow strips of loose cotton, carefully placed around the axis between the leaves, and saturated with tap water. Frequent washing of the electrode contacts and measurements of the electrodes showed that the electrodes and contacts never varied more than one millivolt during the readings. This fact should be remembered when considering the curves of figures 1, 2, 3, and 4 presented here. All measurements were made with the potentiometer and consequently we are not concerned with possible variations in electrical resistance.

Many tips of lateral branches and main axes were studied with care, but all gave the same type of result. In view of this fact the details of only two typical experiments will be given, one upon the apex of a lateral branch and the other one on the apex of the main stem.

External polarity potentials in the apex of a lateral branch

A lateral branch from the lower middle region of a ten year old tree was cut 49.0 centimeters from the apex of the branch. The branch was set up in its original horizontal position. Electrode contacts were made at A, B, C, D, E, and F as indicated in the diagram of figure 1. The lengths of the segments AD, BD, CD, DE, EF, and FG were 32.5 cm., 20.0 cm.,

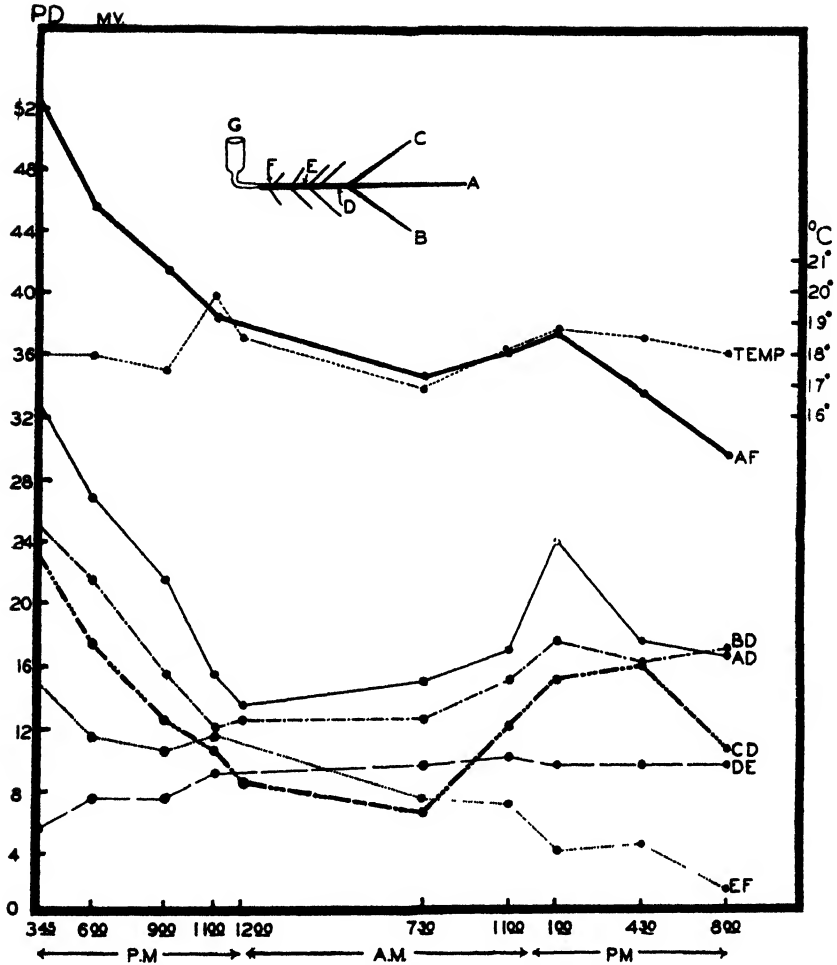


FIG. 1. The diagram at the top of the figure represents the end of a lateral branch. A, B, C, D, E, and F are the points at which the electrode contacts were made. G is a cup contact filled with tap water and inserted into the pith of the stem. The actual lengths of the segments AD, BD, CD, DE, EF, FG were respectively 32.5 cm., 20.0 cm., 19 cm., 6.5 cm., 7.8 cm., and 2.5 cm. Potential differences between the external contacts were made at the times indicated on the abscissa. The preparation was mechanically undisturbed during the experiment. Curve AF shows the magnitude of the P.D. between contacts A and F during the experiment. Calculation shows the ordinates of AF to be equal to the sum of the ordinates of the curves AD, DE and EF. Note that the polarity of AD is greater than BD and CD. Also note that the absolute amounts of the variations of electric polarity in AD, CD and BD are greater than the variations of DE or EF. The room temperature is indicated by the curve at the top.

19.0 cm., 6.5 cm., 7.8 cm., 2.5 cm., respectively. The diameter of the stem at the cut end was 7 mm. A bent glass funnel, G, filled with tap water was inserted to a distance of 5 mm. into the pith at the basal end of the stem. This served as an electrode contact for measurements of radial potentials.

The cut branch was taken to the laboratory and set up at 9:00 A. M. It was left without mechanical disturbance throughout the period of the experiment. The first measurements were taken at 3:45 P. M. the same day and thereafter at intervals as indicated on the abscissa in figure 1. The range of variation of room temperature is given by the upper broken curve. The external P. D. between A and F during the experiment, which lasted twenty-eight hours is given by the curve AF. The P. D. between the contacts at A, and at D is given by curve AD. The curves CD, BD, DE, EF similarly give the magnitudes and variations of the external P. D. between the corresponding contacts.

Inspection of the curves shows that the electric polarity of the main apex AD is at all times greater than that of CD or BD, except at the last reading when the polarities of BD, and AD are practically equal. The polarity of BD, is always greater than that of CD. Experiments on other lateral branches gave similar results. We may therefore conclude that the greater electric polarity of the main axis is a general characteristic of the main axis and corresponds in part to its greater length and dominance in growth.

One of the interesting facts about the curves is that the absolute range of variation of the P. D. is very large and greatest in the apexes AD, BD, and CD. This fact has already been shown in a more convincing manner by experiments on the main axis of the whole tree. See LUND (1), curves in figures 8 and 9, pp. 14-15.

From these observations we conclude that the range of variation of the external electric polarity of a branch or of the main axis of the tree increases even up to the extreme apical end. However, since the apex of the main axis of a normal tree is, under ordinary conditions, always electro-positive to apexes of all lateral branches, LUND (2), the apex of the main stem shows the greatest range of variation in electric polarity. This fact obviously corresponds in some way to the fact of its dominance in growth and range of variation in rate of growth. The interesting problem of this intimate relation of the mechanism of electric polarity to dominance in growth we shall consider in a later paper when a sufficient number of other essential preliminary facts have been presented.

Curve AF is the observed P. D. between contacts A and F. Calculations show that each measurement of P. D. between the contacts A and F is equal to the sums of the P. D.'s between A and D, D and E, and E and F if taken at the same time. This is another illustration of what the writer

has called the "principle of summation of E. M. F.'s" along the main axis. A critical examination of all the facts which establish the validity of this principle will be presented in a later paper because of its evident importance for the phenomena of cell correlation.

The measurements of P. D. between contacts G and F, taken at the same times as those represented by the curves in figure 1 were small and showed that F was electronegative to G, the center of the stem, during the first part of the experiment. This orientation is in agreement with all previous observations on the radial potentials in the Douglas fir. The observed maximum P. D. was 7.5 millivolts. However, in the latter half of the period the P. D. decreased and became inverted, amounting finally to the small value of 3.5 millivolts.

It is of course very probable that cells near, and at some distance from, the cut end were injured and therefore the radial polarity between G and F became modified. It is of interest to note that all the measurements of P. D. between G and the contacts E, D, C, B, A *increased* successively from E to A. In all these measurements G was always *electronegative* to the other points in the external circuit. The curves for these measurements are not given because they bear upon a different problem, namely the internal distribution of the correlation potentials in the stem and apex. A paper to be presented later deals with this question.

External polarity potentials in the apex of the main stem

1. RELATIVE MAGNITUDES OF THE POLARITY POTENTIALS IN THE APEX BEFORE STIMULATION

The apex including the first whorl of a ten year old Douglas fir, was cut off 87 centimeters from the apex and removed to the laboratory. The preparation was placed in an erect position with the basal end immersed in a dish of water. Electrode contacts were attached at the points A, X, Y, Z, and O as indicated in the diagram at the bottom of figure 2. Measurements of the polarity of the segments OZ, ZY, YX, and XA were made at intervals during a period of 27 hours. The typical curves of the electric polarity at three different times during this period are given by curves 1, 2 and 3 in figure 2. Every precaution was taken to prevent the least mechanical disturbance of the preparation during the total 27-hour period when the measurements were taken. The variations in the polarity shown by the curves are therefore not due to mechanical effects, but probably due to temperature variations in the tissues and in the room. However, the latter only varied between 18.9° C. and 19.° C. during the measurements. See temperature curve in figure 3.

The significant fact shown by the curves is that in the unstimulated condition of the apical segment the local electric potentials increase quite

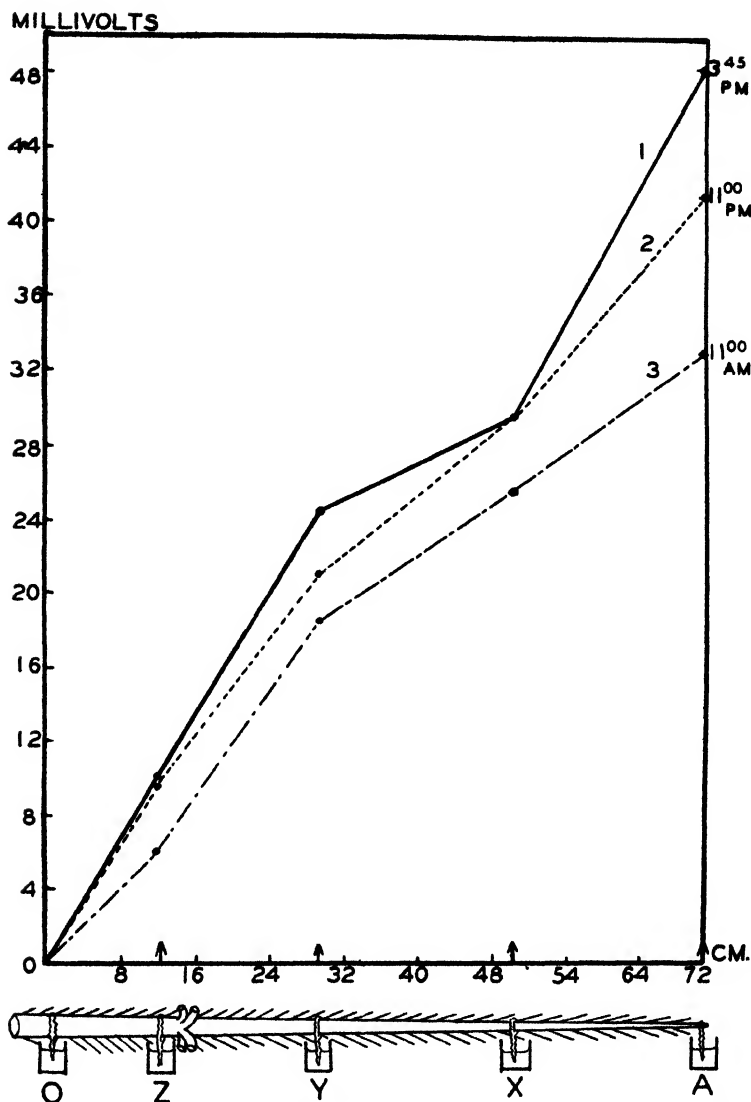


FIG. 2. The curves show the progressive increase in electric potential in the apical region of the main axis of a Douglas fir. (Same preparation as that used for curves in figure 3). The diagram of the main axis at the bottom is drawn to scale. The lengths of the segments OZ, ZY, YX and XA are respectively 12 cm., 17 cm., 21 cm., and 21 cm. The P.D.'s between the contacts are given in the curves. The electric polarities in the absence of mechanical disturbance varies from time to time as illustrated by the slopes of the three curves. These curves should be interpreted in connection with the curves in figure 3.

regularly to a maximum at the growing point. We conclude therefore that the orientation of the electric polarity of the main part of a normal stem as fully described elsewhere, continues to the extreme apical growing point at the apex. The same statement applies to actively growing lateral branches. In the following paper we shall develop the significance of the above facts for the problem of internal distribution of the correlation potentials in the tree.

During the 27-hour period of the above experiment, other measurements were made of the external polarities in this same preparation. The times of the measurements are given on the abscissa in figure 3. Contacts were also made at the beginning of the period at the apexes A, B, C, D, E and F and the base Z of the lateral shoots of the first whorl. These contacts permitted a determination of (1) the magnitude of the electric polarities of the individual segments AZ, BZ, CZ, DZ, EZ, and FZ, and (2) the difference between the polarities of AZ and each one of the lateral shoots BZ, CZ, DZ, etc., under conditions of practical absence of mechanical disturbance in the system. These measurements of polarity are given by the curves in figure 3. The temperature of the room during the period is indicated by the corresponding curve in the figure.

The outstanding fact which is shown by the curves is that the electric polarity of AZ is always much greater than that of BZ, CZ, DZ, EZ, and FZ. Consequently the apex A is always electropositive to all the other tips B, C, D, E, and F. The lateral shoot DZ, consistently showed the largest polarity among the lateral shoots in spite of the fact that its length was less than that of CZ and EZ. Obviously the magnitude of electric polarity in the unstimulated condition is not strictly proportional to length of the tips even at the apex of the tree. The end of curves BZ and FZ illustrate the fact that without apparent mechanical stimulation from an external source the polarity of the apex of a branch may become inverted. The conditions which determine such "spontaneous" variation in polarity are at present unknown and will be considered at a later time. That the inverted polarity of the lateral shoot BZ and FZ during the evening readings at 5 P. M. and 8:30 P. M. was not a permanent condition, is shown by the fact that these shoots as well as all the others exhibited a normal orientation of polarity at 8:00 A. M. the following morning.

It may be that the diurnal rhythm in polarity which occurs outdoors and which, as has been shown, is mainly correlated with the diurnal variation in outdoor temperature, persists to some extent in the apex even when room temperatures are relatively constant. A thorough investigation of this interesting question will be undertaken at a later time. At present we are only concerned with the fact that during apparent absence of mechanical stimulus, the main apex is always electropositive to the apexes of the

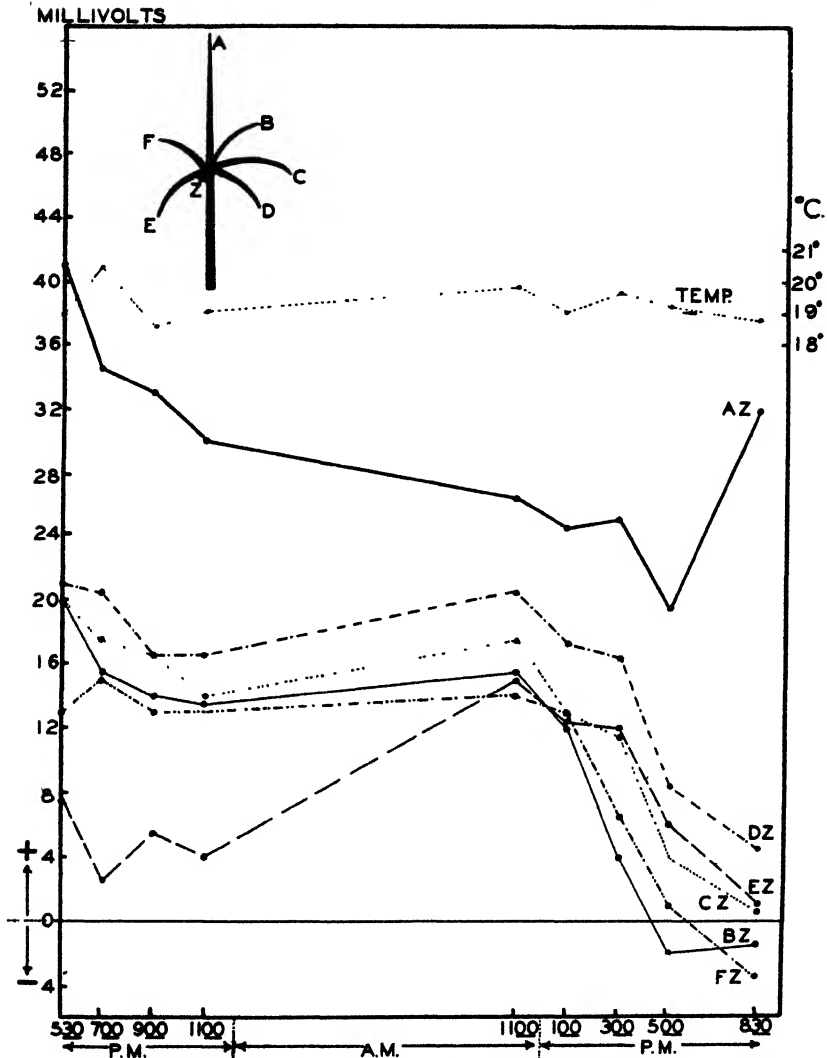


FIG. 3. Curves showing the magnitude and variation of the electric polarities in the growing lateral shoots of the first whorl and the distal shoot AZ of the main axis, in the absence of mechanical disturbance and with a variation of room temperature as shown by the curve-temp. The lengths of AZ, BZ, CZ, DZ, EZ and FZ were respectively 63.0 cm., 30.0 cm., 35.5 cm., 31.5 cm., 35.0 cm., and 30.0 cm. Note that the electric polarity of AZ is the greatest, corresponding to its greater length and dominance in growth. The apex A was therefore electropositive to the apexes B, C, D, E and F, throughout the period of over 27 hours during which the preparation was mechanically undisturbed. Compare these curves of polarity with that in the main axis as shown in figure 2.

lateral shoots of the first whorl, and that this relation is maintained to a variable extent at least over long periods of time if not permanently. The results of many other experiments, which for the sake of economy of space cannot be reported, confirm this general statement.

2. INCREASE, DECREASE, AND INVERSION OF THE NORMAL ELECTRIC POLARITY OF THE APEX BY MEANS OF A MECHANICAL STIMULUS

The results of the foregoing experiments constitute a background of facts for a comparison with the phenomena which result when the growing apex is stimulated mechanically. At 8:00 A. M. the next morning after the last reading in figure 3 was taken, another set of measurements of the polarities of AZ, BZ, CZ, DZ, EZ and FZ was taken. The orientation was normal in all of them and the polarity of AZ was as usual the greatest. Immediately after this set of measurements another set of readings were taken of the P.D. between A and B, A and C, A and D, A and E, and A and F. They showed of course what was to be expected, that the main apex A was electropositive to each one of the apexes B, C, D, E and F. This fact is shown in the first set of readings in the curves of figure 4. At 8:45 A. M. each one of the tips of the lateral shoots was stimulated by a gentle stroke with a pencil. The P.D.'s between A and these tips were then immediately determined. The immediate *rise* in P.D.'s is shown by the curves in figure 4. The curves show that the lateral tips return to their former *relatively* more electropositive condition than that which they showed immediately after stimulation. At 10:30 A. M. the same day the relative polarities had returned approximately to the normal. At 10:32 A. M. the main apex A was stimulated by a gentle blow with a pencil. The curves show that the apex A became electronegative to the tips of the lateral shoots. The recovery of its original polarity is shown by the remaining parts of the curves, readings for which were continued for nearly three hours after application of the stimulus to A. It is evident that mechanical stimulation lowers the electric potential in the region stimulated. With this old and familiar result, in all sorts of irritable tissues we have of course long been acquainted. But the important fact which is brought out by the above simple experiment is, *that the mechanism which operates to produce a continuously maintained but fluctuating electric polarity is in all probability the same fundamental mechanism which is altered by mechanical energy applied to it* in the process of stimulation. To clearly illustrate this linkage in the Douglas fir between continuously maintained electric polarity and the mechanism of irritability is one of the objects of this paper. Similar relations have previously been observed by the writer and MARSH (5) in roots.

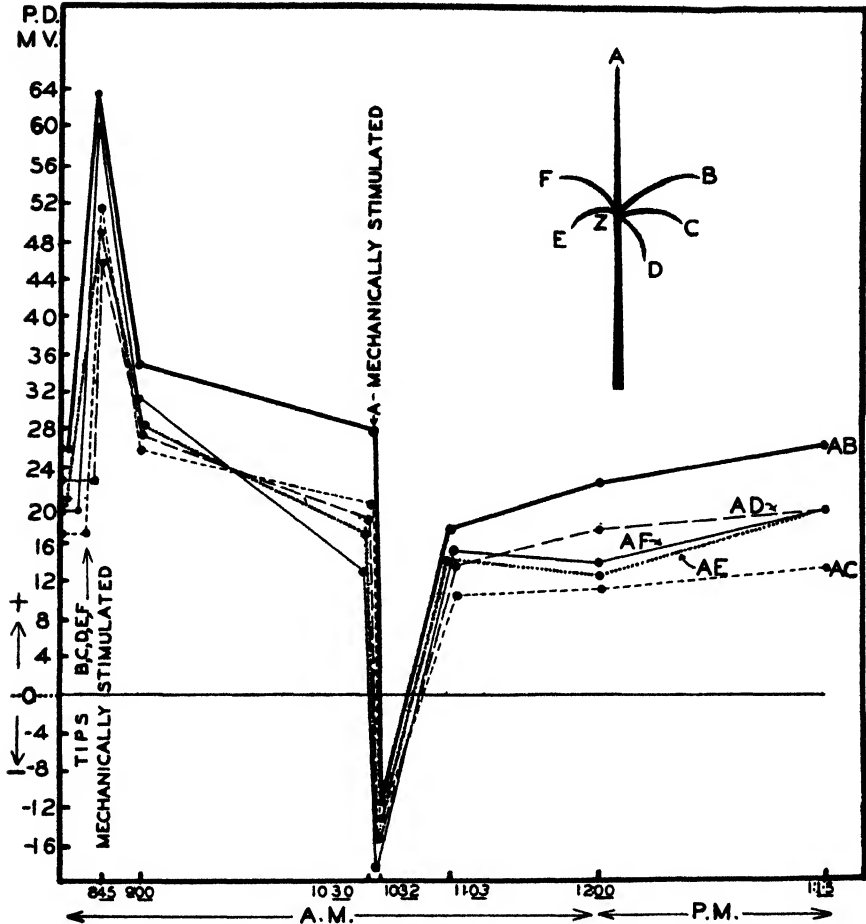


FIG. 4. The curves illustrate the effect of a mechanical stimulus applied to each one of the apices B, C, D, E, and F of the lateral shoots, on the P.D.'s between these apices and the unstimulated main apex A. When recovery from the stimulus of the apices of the lateral shoots has occurred, stimulation of the apex A causes a decrease in its polarity so great that the apices B, C, D, E and F become electropositive to A. The electric polarity of AZ may be temporarily inverted by such a stimulus. Inspection of the first part of the curve shows that the electric polarities of the lateral shoots may also be temporarily inverted by a mechanical stimulus. Recovery of normal orientation of polarities and return to electric "dominance" by A is shown by the last parts of the curves, for A again becomes electropositive to B, C, D, E and F.

If the oxidation-reduction theory of the mechanism of electric polarity and internal correlation currents in polar structures as developed by the writer (4) applies to the Douglas fir in the same way as it has been shown to apply to the onion root, *Obelia* stem, etc., then we are at once given the

interesting opportunity of showing how the flux equilibrium in cell oxidation is altered in the process of stimulation. The effect of stimulation upon the flux equilibrium in the oxidation system of the cell and its relation to electric polarity of the cell was briefly pointed out in an earlier paper by the writer, LUND (4, pp. 279-280). The most recent evidence which indicates the relation between cell oxidation and the nerve impulse is of interest in this connection, *e.g.*, SCHMITT (6).

The decrease and reversal of electric polarity of growing points by mechanical stimuli also raises interesting questions as to the rôle of mechanical stimulation and electric polarity in the development of form of plants and animals. For example, it may often be observed in the forests of the Northwest that two Douglas firs whose axes grow close to one another often form an apparently single symmetrical tree in respect to external form. The above experiments suggest the possibility that mechanical contacts of adjacent growing points bring about a mutual decrease in electric polarity of the stimulated points and consequently a decrease or even inhibition of apical growth.

Summary

1. In the normal unstimulated condition of the Douglas fir, *Tsuga pseudotsuga*, the main apex of a lateral branch maintains an electropositive condition to all points on the branch below it. The main apex is also electropositive in the external circuit, to each one of the apexes of the first pair of lateral shoots. The electropositive condition of the main apex corresponds to its dominance in growth.

2. The usual range of variation in electric polarity in the unstimulated apex under laboratory conditions, is greater than in more basal parts of the lateral branch.

3. The electric polarity previously described, in lower parts of the main axis has been found to continue as a regular increase in electropositive condition up to the growing point of the axis of the stem.

4. The apex of the main axis in the unstimulated condition is always electropositive to the apexes of the shoots of the first whorl. This corresponds to the dominance in growth by the main apex.

5. Spontaneous variations in electric polarity similar to those occurring in roots, occur in all apical growing points of the Douglas fir. The causes of these variations are not at present known.

6. A mechanical stimulus applied to any apex decreases or reverses temporarily its electric polarity. In this manner the P.D. between the main apex and that of any lateral shoot of the first whorl may be (1) increased, (2) decreased or (3) reversed.

7. Since the electropositive condition of an apex is undoubtedly related in part to the physico-chemical process of growth within the apex, it is suggested that mechanical stimulation in nature may alter dominance in the growth processes in a corresponding manner to that by which it alters the electric properties of the growing point.

8. The preliminary simple experiments in the present paper suggest that the physico-chemical mechanism of maintained electric polarity is the same as, or at least is a mechanism which is linked with, the mechanism of irritability of the living cells. This same conclusion also follows from previous work in this laboratory on the electric polarity in roots.

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SEASONAL CHANGES IN TOTAL, SOLUBLE, SOLUBLE-PROTEIN, NON-PROTEIN, AND INSOLUBLE NITROGEN IN CURRENT YEAR'S SHOOTS OF BARTLETT PEAR

A. S. MULAY

(WITH TWO FIGURES)

Not only are proteins very closely connected with the phenomena of life, but unlike other essential constituents of the living cell, they are in all probability characteristic of the animal or plant species in which they are found. The physical properties of the proteins apparently adapt them to the performance of functions essential to the activities of life. Notwithstanding this unique position which these nitrogenous compounds occupy in the plant metabolism, but little is known as to the part they play in the metabolic cycle of the higher plants.

Owing to the difficulties involved in separation and identification of various nitrogenous compounds, earlier investigators could not proceed much beyond the determination of total nitrogen in their material. Recent advances in protein chemistry have made a further study of these nitrogenous compounds possible. OSBORNE's work on proteins in spinach leaves (7) closely followed by a series of papers by CHIBNALL on proteins in leaves of various annuals, including studies on runner bean leaves (3), stimulated interest in the study of nitrogen metabolism of the higher plants by nitrogen fractionation methods. THOMAS (8) was the first to undertake a rather complete nitrogen fractionation of woody tissue like apple twigs.

This work was undertaken to determine the seasonal variations in the nitrogenous compounds in the bark and the wood of the current year's growth in Bartlett pear trees. This paper deals with fractionation of total nitrogen into water-soluble and insoluble nitrogen and further division of water-soluble into soluble protein and non-protein nitrogen. Fractionation of soluble non-protein and insoluble nitrogen will be taken up in later papers.

Material

The samples were taken from twelve 6-year-old Bartlett pear trees grown at the University Farm, Davis, California. These trees were fairly uniform and may be considered as representative trees for their age on a well-managed farm in that locality.

Samples were collected at intervals of approximately one month from September 1927 to January 10, 1929. A representative sample of four to five current year's shoots was collected from each tree, giving a composite

sample of 50-60 shoots. This number, as shown in a previous paper (6), will give results which are fairly representative of the whole group. The total length of the shoots was included in the sample. The dates of collection together with the dry weight of the samples, wood to bark ratio, dry weight as percentage of net weight, and percentage of dry weight extracted with water, are given in table I.

TABLE I
COLLECTION DATA SEPTEMBER, 1927-JANUARY, 1929

DATE COL- LECTED	DRY WEIGHT			DRY WEIGHT		H ₂ O SOLUBLE DRY MATTER	
	BARK	WOOD	WOOD BARK	AS PERCENTAGE OF WET WEIGHT		AS PERCENTAGE OF DRY WEIGHT	
				BARK	WOOD	BARK	WOOD
	<i>gm.</i>	<i>gm.</i>		<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>
Sept. 16	117.6	205.6	1.75	40.5	52.8	27.2	14.5
Oct. 21	130.0	211.0	1.62	45.5	58.9	29.3	16.5
Dec. 7	74.0	115.0	1.55	43.7	59.3	31.8	24.0
Jan. 15	124.0	149.0	1.20	45.2	57.4	30.0	23.2
Feb. 11	147.0	181.0	1.23	43.1	56.5	27.2	23.2
Mar. 18	97.0	135.0	1.39	39.1	55.4	30.0	13.8
Apr. 18	78.0	99.0	1.27	32.5	45.0	28.3	15.5
May 13	102.0	143.0	1.40	32.6	53.1	32.2	17.0
June 24	122.0	138.0	1.13	39.0	44.0	28.8	18.5
July 15	166.0	199.0	1.20	40.8	49.8	28.3	15.2
Aug. 19	129.0	176.0	1.36	42.5	53.6	29.3	18.3
Sept. 19	151.0	197.0	1.30	44.4	55.4	29.2	18.5
Oct. 15	159.0	216.0	1.36	45.4	58.4	32.0	20.2
Nov. 17	162.0	202.0	1.25	43.5	57.3	26.2	18.2
Jan. 18	170.0	186.0	1.09	45.2	55.0	28.2	18.2

Though an effort was made to collect representative samples, the proportion of weak shoots on the tree increased after each collection, due to the natural tendency to take larger shoots. As a result 1928 shoot growth was not as vigorous as it would have been otherwise. Comparison of the wood to bark ratios (table I) for corresponding dates, in 1927 and 1928 brings out the fact that the 1928 wood was not as well developed as that in 1927. This will probably explain the differences in the results in these two years.

As a rule collections were made in the forenoon, the shoots cut into suitable lengths, put into tight metal cans, and carried to Berkeley without delay. The samples were immediately weighed, separated into bark and

wood, and dried in a ventilated oven at 55° C. The samples were generally in the oven within 12–14 hours, and at the latest within 24 hours, from the time they were picked and were oven-dry within the next 12 hours. Changes in the nitrogen distribution due to proteolytic activity during this time are negligible as shown by LINCOLN and MULAY (5). The dried samples were ground first in a Wiley mill and then in a ball mill until they were reduced to 100 mesh size. The samples thus dried and powdered were preserved at room temperature in screw top glass jars.

Methods of analysis

Total nitrogen (T. N.).—As it was not possible to demonstrate the presence of nitrates in the material used, all nitrogen determinations were made by the simple Gunning method for the determination of total nitrogen as described in the Official Methods of the A. O. A. C. (1). Nitrogen determined on an oven-dry sample, prepared as described before, gives total nitrogen in the sample.

The total nitrogen in the sample was divided into two parts, soluble and insoluble, by extracting the sample with water. The soluble nitrogen in its turn was again divided into protein and non-protein portions by colloidal iron precipitation.

Extraction.—Three 20-gram portions of an oven-dried sample were each mixed with 200 cc. of cold water in 500-cc. Erlenmeyer flasks and agitated for two hours with a mechanical stirrer. Extraction with cold water at room temperature was preferred to extraction with water at 40° C. (9) as it is less removed from the temperature conditions in the living tree. The resulting mixture was centrifuged and the supernatant liquid decanted into a container. The residue was suspended in about 50 cc. of water, centrifuged, and the washings added to the extract, the process being repeated three times. The extracts and washings from the three portions were combined and made up to 1,000 cc. This procedure gave very complete extraction of the water soluble portion.

Soluble nitrogen.—Total soluble nitrogen was calculated from the total nitrogen determination on an aliquot of the extract.

Soluble protein nitrogen.—Soluble proteins were precipitated from the remainder of the extract by colloidal ferric hydroxide as described by THOMAS (8) with the difference that the extracts were brought to pH 4 for reasons discussed elsewhere (5). This divides the soluble nitrogen into two parts, non-protein in the filtrate, and protein in the precipitate. The precipitate was separated from the filtrate by centrifuging and decanting, washed three times with water and then discarded. The washings and filtrate were combined and made up to 1,000 cc. The soluble proteins were

determined by the difference between nitrogen determinations before and after the precipitation of the proteins.

Non-protein nitrogen.—Nitrogen determination on an aliquot of the filtrate gives the value for non-protein nitrogen.

Insoluble nitrogen.—The residue remaining after water extraction was dried, weighed and ground to a fine powder. Nitrogen determination on a portion of this residue gives values for insoluble nitrogen.

Protein nitrogen (P. N.).—Insoluble nitrogen plus soluble-protein nitrogen is taken as the measure of protein nitrogen.

Proteins.—Protein nitrogen $\times 6.25$.

All determinations were run in duplicate except those on total, insoluble, and amino nitrogen, which were run in triplicate. In all cases the duplicates were within 3 per cent. of the mean and within 5 per cent. of each other. However, the majority of the duplicates were within one per cent. of each other. Accuracy of the methods was further tested by comparing the calculated values for insoluble nitrogen with those obtained by actual determinations. It was found that all the actual values but one were within 5 per cent. of the calculated values, the majority of them being within 3 per cent.

Results

Tables II and III show seasonal changes in total, soluble, soluble-protein, non-protein, and total protein nitrogen fractions in bark and wood respectively. The same results are graphically represented in figures 1 and 2.

Discussion

TOTAL NITROGEN

Total nitrogen in the bark (fig. 1) fluctuates between 0.7 and 1.1 per cent. of the dry weight of the bark. Shoots of 1927 begin to increase in nitrogen at the end of October and reach their peak, 1.1 per cent., in December. They remain at this level until February and begin a sharp fall in March when new shoots begin to grow. The activities of new growth make a great demand on nitrogen reserve of the 1927 shoots. As a result total nitrogen in the bark of the 1927 shoots falls very rapidly to 0.7 per cent. in May, *i.e.*, after two months of growth of the 1928 shoots. Shoots of 1928 have a high nitrogen level in May, which falls during the period of active growth, most probably because it cannot keep pace with the increase in complex carbohydrates like cellulose, and lignin. It is at its lowest level, 0.77 per cent., about August, the end of the active growing period, then it increases until November and remains at this level the rest of the season.

Total nitrogen in the wood, except in very young wood where it reaches as high as 1.1 per cent. (fig. 2), varies from 0.4 to 0.7 per cent. of the dry

TABLE II
SEASONAL CHANGES IN TOTAL, SOLUBLE, SOLUBLE PROTEIN, NON-PROTEIN, AND INSOLUBLE NITROGEN
BARK

DATE COL- LECTED	AS MG. PER 100 GM. OF DRY WEIGHT					AS PERCENTAGE OF TOTAL NITROGEN				SOLUBLE PROTEIN N AS PER- CENTAGE OF SOLU- BLE N	
	TOTAL N	SOLUBLE NITROGEN		INSOLU- BLE N	TOTAL PROTEIN N	PROTEIN (PROTEIN N \times 6.25)	TOTAL SOLUBLE N	SOLUBLE PROTEIN N	TOTAL PROTEIN N		
		TOTAL	NON- PROTEIN								
											mg.
Sept. 16	809	120	94	25	717	742	4640	14.8	3.1	88.3	21.0
Oct. 21	818	169	106	63	667	730	4560	20.7	7.8	87.1	37.7
Dec. 7	1114	218	165	53	901	955	5960	20.5	5.7	85.2	27.8
Jan. 15	1112	164	119	45	935	980	6125	14.7	4.0	89.3	27.2
Feb. 11	1120	181	132	50	942	991	6195	16.2	4.5	88.3	27.8
Mar. 18	1022	255	194	61	733	794	4960	25.0	6.1	81.1	24.4
Apr. 18	784	185	151	35	591	626	3910	23.6	4.4	80.8	18.6
May 13	714	146	105	41	558	599	3750	20.5	5.7	85.2	27.8
June 24	826	179	136	43	611	654	4090	21.7	5.3	85.6	24.4
July 15	767	216	137	78	570	648	4055	28.2	10.3	82.1	36.6
Aug. 19	771	208	112	96	571	667	4165	27.0	12.5	85.5	46.3
Sept. 19	861	243	124	119	636	755	4720	28.3	13.9	85.6	49.2
Oct. 15	944	260	124	136	666	802	5000	27.6	14.4	86.8	52.3
Nov. 17	1032	162	124	38	893	931	5810	15.7	3.7	88.0	23.6
Jan. 18	1040	201	95	106	799	904	5655	19.4	10.2	90.8	52.6

TABLE III
SEASONAL CHANGES IN TOTAL, SOLUBLE, SOLUBLE PROTEIN, NON-PROTEIN, AND INSOLUBLE NITROGEN
WOOD

DATE COL- LECTED	AS MG. PER 100 GM. OF DRY WEIGHT					AS PERCENTAGE OF TOTAL NITROGEN				SOLUBLE PROTEIN N AS PER- CENTAGE OF SOLU- BLE N
	TOTAL N	SOLUBLE NITROGEN			TOTAL PROTEIN N	PROTEIN (PROTEIN N \times 6.25)	TOTAL SOLUBLE N	SOLUBLE PROTEIN N	TOTAL PROTEIN N	
		TOTAL	NON- PROTEIN	PROTEIN						
Sept. 16	mg. 470	mg. 213	mg. 174	mg. 39	mg. 305	mg. 1909	per cent. 45.5	per cent. 8.5	per cent. 63.0	per cent. 18.7
Oct. 21	455	213	177	36	272	1700	46.9	7.9	61.0	16.8
Dec. 7	532	262	214	48	326	2038	48.1	8.1	59.7	16.8
Jan. 15	570	277	234	43	333	2082	48.6	7.5	58.9	15.4
Feb. 11	547	234	206	28	337	2108	42.8	5.1	62.3	11.9
Mar. 18	622	325	279	46	350	2185	52.3	7.5	55.2	14.3
Apr. 18	437	174	143	31	289	1805	39.8	7.0	67.2	17.6
May 13	426	130	113	17	316	1973	30.5	3.9	73.4	12.8
June 24	669	257	232	25	418	2610	38.5	3.8	65.3	9.9
July 15	717	358	327	31	376	2330	49.9	4.3	54.4	8.6
Aug. 19	678	363	330	33	349	2181	53.5	4.7	51.2	8.8
Sept. 19	708	379	336	43	382	2383	53.5	6.0	52.5	11.2
Oct. 15	734	398	345	53	378	2362	54.3	7.2	52.9	13.3
Nov. 17	703	362	309	53	386	2415	51.6	7.6	56.0	14.7
Jan. 18	630	327	280	47	363	2268	51.2	6.8	55.6	13.3

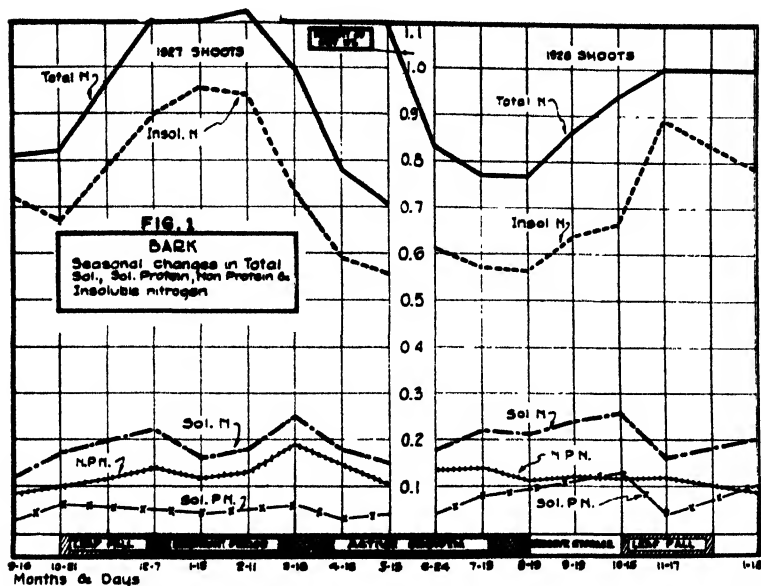


Fig. 1. Seasonal changes in total nitrogen of the bark of the Bartlett pear shoots.

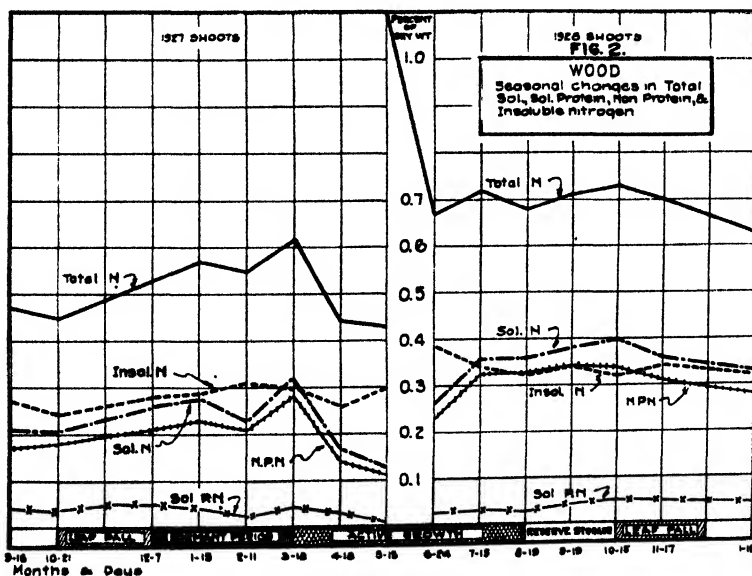


Fig. 2. Seasonal changes in total nitrogen of the wood of Bartlett pear shoots.

weight. This is only about 60 per cent. of that in the bark. The seasonal changes in the total nitrogen in the wood do not look as marked as those in the bark (fig. 1), when absolute amounts are compared but on a percentage basis the magnitude of the changes is just about the same in both cases. In the 1927 shoot wood there is a rise in total nitrogen from 0.45 per cent. in October to 0.62 per cent. in March, and then a pronounced drop in April which is probably due to translocation of these substances to actively growing 1928 shoots. Nitrogen of shoot wood of 1928, amounting to 1.0 per cent. in May, falls sharply in June and then changes but little throughout the season except for a gradual drop from October to January. Shoots of 1928 are much higher in total nitrogen as compared with those of 1927. This is believed to be due to delay in wood fibre formation in 1928 shoots, because of their slower growth, reasons for which are discussed under material.

HOOKE (4), working with apple spurs, BUTLER, SMITH and CURRY (2), and THOMAS (9), working with one-year-old apple branches, find similar fluctuations in total nitrogen in their material, though none of them separated bark from wood.

Total nitrogen shows a steady rise in both bark and wood of 1927 shoots from October to December. The bark nitrogen shows a rapid fall in February, March, and April, when flowers and leaves unfold and new shoots make rapid growth. The wood nitrogen falls sharply only in March and April. The bark of 1928 shoots has 1.0 per cent. nitrogen in May which falls off to 0.76 per cent. during the period of rapid growth and then follows the same trend as 1927 bark, except that the rise starts in August. Wood of 1928 falls from 1.1 to 0.68 per cent. in the course of a month of rapid growth from May to June and then fluctuates but little until it falls to 0.62 per cent. in January.

SOLUBLE NITROGEN

TOTAL SOLUBLE NITROGEN.—The soluble nitrogen in bark constitutes about 20 to 30 per cent. of the total nitrogen (fig. 1). In shoots of 1927 it shows the same trend as total nitrogen in bark in as much as both rise from October to December and both fall when new shoots begin to grow. However, it shows two maxima, one in December and the other in March. In December insoluble nitrogen seems to increase at the cost of soluble. Judging from the slopes of the curves for total, soluble, and insoluble nitrogen between February and March, the rate of proteolysis is much faster than the rate of transport at this time and there is a temporary accumulation of soluble nitrogen which gives it its second maximum. Beginning in the latter part of March when leaves have started to open,

the rate of transport presumably exceeds the rate of proteolysis and there is a rapid fall in both soluble and insoluble nitrogen. In shoots of 1928 soluble nitrogen shows a rise until October then a sharp drop in November and a rise again in January.

Soluble nitrogen in wood (fig. 2), which constitutes about 50 per cent. of the total nitrogen, follows the same trend as total nitrogen. Wood of 1927 shows a small rise from 0.2 per cent. in October to 0.28 per cent. in January, a small drop in February, to 0.23 per cent. followed by a marked rise to 0.32 per cent. in March and then a sharp drop to 0.17 per cent. in April and 0.13 per cent. in May. Wood of 1928 shows a marked rise from 0.26 per cent. in June to 0.36 per cent. in July, then a slow rise to 0.4 per cent. in October and a drop to 0.33 per cent. in January.

Soluble nitrogen both in bark and in wood shows a rise in autumn and early winter and a rapid fall in spring when new shoots start their growth. It constitutes about 20 per cent. of the total nitrogen in the bark and about 50 per cent. of that in the wood. On a dry weight basis it is about 0.2 per cent. in the bark and varies between 0.15 and 0.4 per cent. in the wood.

SOLUBLE PROTEIN NITROGEN.—In bark the soluble-protein fraction (fig. 1) forms about 6 to 12 per cent. of the total nitrogen and about 0.05 to 0.1 per cent. of the dry weight. In the bark of 1928 it starts at a low level in June, increases steadily during growth until October, drops sharply in November, and rises again in January. The sharp drop in November, 1928, may be due to some unknown factor which prevented the extraction of the soluble protein (5). In the shoots of 1927 it fluctuates but little except for a sharp rise in October and a sharp drop in April. The sharp rise from September to October is similar to that between November and January in 1928, and may have some significance though the time of its occurrence is shifted by two months. In wood soluble protein forms about 8 to 9 per cent. of the total nitrogen and about 0.04 to 0.06 per cent. of the total dry weight. In the wood of 1927 soluble proteins fall in January and February, rise in March, and fall again till May (Fig. 2). In the wood of 1928 soluble proteins show very little change.

NON-PROTEIN NITROGEN.—Total non-protein nitrogen in both bark and wood shows, with the exception of the bark of the 1928 shoots, the same seasonal fluctuations as soluble nitrogen (figs. 1 and 2). It forms about 50 to 90 per cent. of the soluble nitrogen in the bark and 90 to 95 per cent. of it in the wood.

INSOLUBLE NITROGEN

In bark, insoluble nitrogen constitutes about three-fourths of the total nitrogen and all the changes in total nitrogen seem to be mostly due to the changes in this fraction (fig. 1). In wood, insoluble nitrogen accounts for

about half of the total nitrogen and unlike in bark has no marked effect on total nitrogen fluctuations (fig. 2) which correspond closely to the changes in soluble nitrogen.

Summary

Seasonal fluctuations in total and soluble nitrogen and its fractions in bark and wood are summarized in table IV.

TABLE IV
SEASONAL FLUCTUATIONS IN NITROGEN SUMMARIZED

	AS PERCENTAGE OF DRY WEIGHT				SOLUBLE N AS PERCENTAGE OF T. N.		
	TOTAL N	SOLUBLE NITROGEN			TOTAL	NON-PROTEIN	PROTEIN
		TOTAL	NON-PROTEIN	PROTEIN			
	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>
Bark	0.8-1.1	0.18-0.22	0.1-0.15	0.05-0.1	20-25	15-20	5-10
Wood	0.5-0.7	0.25-0.35	0.2-0.3	0.04-0.06	40-50	35-45	4-8

New growth starts with high total nitrogen in the bark which falls as the growth proceeds. After the slowing down of growth the total nitrogen increases, reaches a maximum in winter, and falls again when new buds begin to open and draw on the reserves in these shoots. Insoluble nitrogen is mainly affected in these seasonal changes.

The total nitrogen in the wood shows changes somewhat similar to those in the bark. However, unlike bark, changes in soluble nitrogen are mainly responsible for the seasonal fluctuations in wood.

Soluble proteins, which form only a small fraction of the total nitrogen, both in the bark and in the wood, show an increase in late summer and autumn, and a fall in later winter and spring.

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STUDIES ON PARENCHYMATOUS AND VASCULAR PLANT TISSUES, SOME ANALYTICAL AND SPECIFIC GRAVITY DATA¹

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Introduction

Recent work on the industrial utilization of agricultural wastes has included a study of methods available for the possible utilization of cornstalks. Such material differs from wood, which consists mainly of xylem, in the presence of an appreciable amount of parenchyma. Most of the weight of the mature stalk is due to cell wall material. The marked difference in properties exhibited by the parenchymatous and vascular tissues of the mature cornstalk indicates that it may be desirable to utilize these tissues for different purposes or to subject them to different treatments. The selection of the most efficient method for the utilization of these tissues will depend on a more complete knowledge of them. In view of these facts, a comparison of the cell wall materials of parenchymatous and vascular tissues is of practical as well as of botanical interest.

If the epidermal layer is disregarded, the entire stalk may be said to consist of parenchymatous and vascular tissues. The pith or fundamental parenchyma is a simple tissue, composed of large, isodiametric, thin walled cells. The vascular material, on the other hand, includes two complex tissues, phloem and xylem, and two simple tissues, sclerenchyma and parenchyma. The sclerenchyma, which consists of heavy walled cylindrical cells with diameters many times less than the diameter of the average pith cell, is structurally similar to the tracheids, the most abundant of the xylem cells. For this reason, the term xylem will be used to include all cells of this type. Since the parenchyma and phloem constitute only a small part of the total weight of the vascular tissue, the properties of this material may be regarded as essentially due to xylem.

According to the generally accepted view, the cell walls of parenchyma and xylem of a given plant are chemically different. This conception is based on the results of microchemical tests and staining reactions. Such methods have the disadvantage of being entirely non-quantitative. Furthermore, recent work has questioned the reliability of such methods (3, 5).

Various macrochemical methods have been developed for the estimation of cell wall constituents. Most of these methods are based on the assump-

¹ Contribution from the Chemistry Department of Iowa State College.

tion that certain solvents, such as acids, alkalies, etc., have a selective action on the constituents of the wall. Actually, it seems unlikely that these reagents either entirely remove or leave a residue consisting of any one constituent. Furthermore, the treatment with such solvents is probably in many cases sufficiently drastic to induce chemical changes in the wall constituents. In this case, the product estimated as a wall constituent is in reality a reaction product of one or more of these constituents. The purely arbitrary character of such procedures is evidenced by the fact that the different methods for the estimation of any one constituent seldom give identical results. Other methods are based on the estimation of some characteristic group, as for example, the determination of pentosans as furfural phloroglucide. In view of the fact that the exact relation of such groups to the molecule of the constituent is usually unknown, such methods are also open to criticism. Although the proposed macrochemical methods are open to these criticisms, they offer one of the most satisfactory methods at present available for the examination of plant tissues.

By the use of such methods, PETERSON and HIXON (8) analyzed hand separated parenchyma and vascular fractions of the mature cornstalk for the wall constituents known to be present, namely: lignins, pentosans, and cellulose. The analytical results for the separated tissues and for the total stalk were practically the same. WILEY (12) had previously obtained analytical results on such fractions which were more nearly in agreement than would be expected from the previously mentioned conception of cell wall chemistry. WILEY (12) and BURKE (2) reported similar results for the pith and woody fractions of the corncob, a modified stem structure. Such results suggest that the cell walls of parenchyma and vascular material are more nearly alike chemically than has been supposed. The present work was undertaken to furnish additional evidence for or against the hypothesis that these tissues are chemically similar.

Analytical data of the type obtained by PETERSON and HIXON (8) gives no information concerning the nature of the association of the constituents in the cell wall. It is possible that the same substances may be present in the same proportion by weight, and yet be so associated as to produce a chemical difference in the walls. Since chemically different substances are characterized by a difference in physical constants, the desirability of supplementing the analytical data with measurements of such constants is apparent. For this reason, analyses and specific gravity measurements were made on parenchyma and vascular material from the stems of the following plants: (a) ordinary field corn (*Zea mays*); (b) and (c) two genetically pure strains of corn (*Zea mays*),² differing only with respect

² These tissues were kindly supplied by Dr. FISK GERHARDT, of the Chemistry Section of the Iowa Agricultural Experiment Station, Ames, Iowa.

to the gene controlling rigidity, one being weak and prostrate, the other strong and erect; (d) sugar cane (*Saccharum officinarum*);³ (e) sorghum cane (*Sorghum vulgare*); and (f) Jerusalem artichoke (*Helianthus tuberosus*).

Method

PREPARATION OF THE TISSUES

The preliminary treatment of the plant materials consisted of separation into different tissues or combinations of tissues, grinding, and extraction with such solvents as were expected to leave a residue consisting of relatively pure, unchanged cell wall material. Internodes of stems of the first five plants named above were separated, by peeling with a sharp knife, into two portions: (a) the outer shell of the stalk, consisting of the epidermis and the peripheral vascular bundles, and (b) the central cylinder of the stalk, consisting of the inner vascular bundles and the pith or fundamental parenchyma. The separation of the sugar cane into shell and central cylinder was immediately followed by extraction with acetone to reduce the sugar concentration to such a point that the tissue could be dried by evaporation in air. The central cylinders of the field corn and sugar cane were further separated into vascular bundles and pith. This was accomplished by carefully pulling out the bundles from cylinders which had been softened by soaking and boiling in water. The Jerusalem artichoke stem was split lengthwise and the inner cylinder of purely parenchymatous tissue was scraped out.

Unless otherwise indicated, all tissues were ground to pass a 60-mesh screen. Extraneous materials were removed by exhaustive extraction with water and alcohol. The tissues were air dried before extraction with a new solvent. The final alcoholic extractions of sorghum and artichoke tissues were carried out in Soxhlets. This seemed necessary, to remove the large amount of coloring matter present in these tissues. All specific gravity measurements and analyses were made on air dried tissues.

SPECIFIC GRAVITY MEASUREMENTS

Specific gravity measurements were made by displacement of alcohol. The specific gravity values were calculated from the following formula:

$$\frac{S \times D_1}{(A_1 - A)} = D$$

where A_1 is the weight of alcohol held by the pycnometer when no sample is present, A the weight of alcohol held when the sample, S , is present;

³ These tissues were kindly supplied by the Louisiana Sugar Experiment Station, Baton Rouge, Louisiana.

D_1 the specific gravity of the alcohol, and D the specific gravity of the tissue.

A 100-cc. pyknometer fitted with a ground glass stopper with a capillary outlet was used. The pyknometer was calibrated to 0.0001 cc. at the temperature at which the measurements were to be made. For all measurements, the pyknometer was completely filled with liquid and was brought to constant temperature, by immersing up to its neck for thirty minutes in a water thermostat where the maximum temperature variation was not over 0.02°C . The joint of the pyknometer was protected from any alcohol which might overflow from the capillary outlet, by placing a tight fitting collar of filter paper around the stopper above the joint. Before weighing, the surface of the pyknometer was dried and cleaned by wiping with a cloth moistened with alcohol and ether. The samples of tissue used were of such size that in the dry condition a sample filled one-fourth to one-third the volume of the pyknometer. Samples of greater size interfered with subsequent manipulations. The sample was well covered with alcohol of known specific gravity, at least twenty-four hours before the measurement was to be recorded, to allow for penetration of the tissue. After penetration had occurred, as evidenced by the appearance and the sinking of the tissue, the pyknometer was carefully rotated in such a way that its entire contents were set in motion. This was necessary to remove air bubbles which lodged in the mass of tissue. Check readings were obtained several times on each sample by bringing to constant temperature, readjusting the alcohol level, and weighing. The contents of the pyknometer were agitated between readings to make certain that all the air had been removed.

The ground glass joint on most pyknometers must be reground with fine emery and rouge to make them alcohol tight. When this is done, the stopper is frequently pushed into the neck of the pyknometer to such an extent that a small groove is formed at the top of the joint, where the diameter of the neck of the pyknometer is greater than that of the stopper. It is necessary to remove this by grinding down the neck of the pyknometer until a right angle point of contact between stopper and pyknometer is obtained, to prevent capillarity drawing alcohol from the interior of the flask.

ANALYSES

No attempt to make a complete analysis of the various tissues was made. Moisture, pentosans, lignins, and, if the supply of material permitted, cellulose analyses were made on all tissues. The lignin analyses were made by the 72 per cent. sulphuric acid method, using the procedure recommended by SCHORGER (11). Pentosans were determined as furfural phloroglucide (1). The cellulose pulp was prepared by a modification of

the DE VAIN's process. The pentosan content of the pulp was determined and calculated to per cent. of original stalk. By subtracting this figure from the per cent. of original stalk obtained as pulp, a value for cellulose by difference was obtained.

Results

The experimental data are shown in table I. The values given for specific gravity are averages of values obtained on two or more samples of tissue. The value for each sample is in turn an average of at least two and more often three readings on that sample. The analytical values are averages of two or three determinations.

The specific gravity, pentosan, and cellulose values for the parenchymatous fractions are approximately equal to the corresponding values for the vascular fraction in ordinary field corn, the two genetically pure strains of corn, sorghum cane, and sugar cane. The lignin content of the two fractions was also found to be the same in those tissues where the lignin determinations were run at room temperature, namely: those of field corn, the two genetically pure strains of corn, and sorghum cane. PETERSON (7) recently reported that a difference in lignin content was found in the two tissues of the cornstalk when the analyses were run at the temperature of the ice box. Since his values by this method more nearly agree with those obtained by the WILLSTÄTTER hydrochloric acid method, he has suggested that more nearly correct results are probably obtained at ice box temperature than at room temperature. A similar difference was observed in the lignin content of parenchymatous and vascular material of sugar cane when the analyses were run at ice box temperature. No analyses were made at room temperature due to an insufficient supply of material. The unexplained effect of temperature on lignin analyses suggests that lignin content so determined is of doubtful value in a comparison of plant tissues. These data are neither extensive enough, nor is the method sufficiently refined to warrant the statement that these tissues are chemically identical. It does seem to indicate that they are more nearly alike chemically than is generally supposed. The objection may be raised that in most cases pure tissues have not been dealt with. For example, it may be suggested that the vascular tissue of the cornstalk consists of a mixture of tissues whose cell walls are decidedly different chemically, but which are present in such proportions that the average specific gravity and composition, as shown by such methods of analysis, equal that of the pith, a purely parenchymatous tissue. At present no experimental proof that such is not the case can be offered. However, it seems unlikely that such an averaging occurs in all the plants mentioned above.

TABLE I

SPECIFIC GRAVITY AND COMPOSITION OF PARENCHYMA AND VASCULAR CELL WALL MATERIAL¹

PLANT	TISSUE	SPECIFIC GRAVITY AT 25° C.	LIG-NINS	PENTO-SANS	CELLULOSE PULP	PENTO-SANS IN PULP	CELLULOSE BY DIFFERENCE
			<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>
Corn ^a	Pith ^d	1.52 ^e	32.0 16.5 ^h	27.7	50.1	12.2	37.9
	Inner vascular bundles ^e	1.515 ^e	35.2 22.5 ^h	26.4	50.2	13.1	37.1
	Shell	1.52 ^e	33.5 25.2 ^h	25.9	55.9	16.6	39.3
Corn, genetic strain 201, weak ^b	Central cylinder	1.515	"	28.8			
	Shell	1.515	23.1	27.2			
Corn, genetic strain 201, strong ^b	Central cylinder	1.51	"	29.5			
	Shell	1.52	24.65	29.3			
Sugar cane ^c	Pith ^d	1.50	18.4 ^h	32.4			
	Inner vascular bundles ^e	1.49					
	Shell	1.52	25.4 ^h	30.7			
Sorghum cane	Central cylinder	1.503	21.7	31.1			
	Shell	1.502	23.0	33.4			
Jerusalem artichoke	Pith	1.540	10.1	25.6	48.5	5.6	42.9
	Shell	1.406	23.9	22.6	53.2	11.4	41.8

¹ Analytical results calculated to percentage of oven-dry (105° C.) samples.^a The analytical data for corn are those listed by PETERSON and HIXON (8).^b This tissue and the analyses for pentosans on it were kindly supplied by Dr. FISK GERHARDT of the Chemistry Section of the Iowa Agricultural Experiment Station, Ames, Iowa.^c This tissue was kindly supplied by the Louisiana Sugar Experiment Station, Baton Rouge, Louisiana.^d This tissue could not be ground to pass a 60 mesh screen in the mill used. When the tissue reached its maximum state of fineness it was removed from the upper part of the mill and used without further subdivision.^e The vascular bundles were cut into $\frac{1}{8}$ to $\frac{1}{4}$ inch lengths with the scissors and were not ground in the mill.^f This value was determined at 29° C.^g An insufficient supply of this material prevented the determination of its lignin content.^h This analysis was run at the temperature of the ice box.

The parenchymatous and vascular material of the Jerusalem artichoke differ both in specific gravity and in lignin content. Even here, however, the results on pentosans, cellulose, and even on lignin, indicate that these tissues are not totally dissimilar. Although the specific gravity and lignin values obtained on the parenchyma and vascular material of the Jerusalem artichoke might suggest that tissues of different percentage composition are characterized by a difference in specific gravity, no generalization of this import is warranted due to the doubtful value of lignin analyses referred to above and to the fact that some tissues having approximately equal specific gravity show a difference in lignin content as great as that reported for Jerusalem artichoke (table I).

If parenchymatous and vascular cell wall material are not greatly different chemically, some other factor or factors must be largely responsible for the difference in properties exhibited by these tissues. Both microscopic examination and comparison of apparent and actual specific gravities indicate that their original mechanical subdivision is quite different. A greater surface of cell wall material is exposed in the pith, which has the smaller apparent specific gravity and which consists of large, thin walled, isodiametric cells, than in the vascular tissue, which consists mainly of heavy walled cylindrical cells with diameters many times less than that of the average pith cell. From generalizations regarding surface phenomena in colloids, it is to be expected that the pith will be more readily acted upon by chemical reagents. The facts are in accord with this prediction.

PETERSON (7) has found that thermophilic bacteria ferment the pith of the cornstalk more rapidly than the vascular shell when both have been ground to pass a 60 mesh screen. Since such a fermentation is a surface reaction, this difference in ease of biological attack is to be expected. This worker has also found that vascular material which has been ground in a colloid mill ferments more rapidly than pith ground to pass a 60 mesh screen. This lends further support to the idea that many of the differences in properties shown by parenchyma and vascular tissue are largely due to the original mechanical subdivision of the tissues.

A difference in staining reaction is characteristic of parenchyma and xylem. If the cell walls of these tissues are chemically similar and have the same specific gravity, this difference must be explained in some other way than as the result of a difference in either of these properties. It seems probable that this apparent difference in staining reaction may be partly an optical effect produced by different masses of the same material. This is comparable to the fact that a spool of thread appears darker than a single strand of the thread or than a piece of fabric woven from it.

The difference in properties exhibited by wallboards from parenchymatous and vascular tissues (6) may probably also be explained as the re-

sult of the original mechanical subdivision of the tissues. DUNLAP (4) has suggested that the porosity of various woods determines their insulating properties. He defines porosity as the ratio,

$$\frac{(\text{the volume of a block of wood}) - (\text{the volume occupied by the cell wall material})}{(\text{the volume of a block of wood})}$$

Since the air is a poorer conductor of heat than cell wall material, the more porous tissue is the better insulating material. If the two tissues of the cornstalk are chemically similar, DUNLAP's hypothesis may be used to explain differences in insulating properties shown by these boards. Furthermore, since the cell walls of these tissues, as shown by the analytical (10) and specific gravity data (9) (4) do not seem to differ greatly from wood cell walls, any difference in insulating properties shown by these tissues and by woods may probably be attributed to their different porosities. The limiting apparent specific gravity of boards prepared from the two tissues of the cornstalk will be the same, namely 1.52. The insulating power of such boards would be identical. Due to the great resistance to loss of identity shown by the cells of these tissues, even in pressure boards, the chance that boards of this limiting density will be prepared, save by the addition of some more dense material, is slight.

In view of the fact that the two tissues of the stalk yield the same substances in at least roughly the same proportions, the extent to which they may be practically utilized for the same purpose will probably depend on the extent of the influence of the cellular structure on the properties of the product. Even where the tissues are to be utilized for the same purpose, the difference in reaction rate exhibited by these materials suggests that it may be desirable to subject them to treatments of different intensity.

Summary

Specific gravity and analytical data on parenchymatous and vascular tissues of a number of plants have been presented. The probable need for a revision of the general conception of the chemistry of the cell walls of these tissues has been suggested. Mention has been made of the importance of the effect of the original mechanical subdivision of tissues on their properties.

The writer wishes to express her appreciation to Dr. R. M. HIXON for his assistance and encouragement during the course of this investigation.

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PERMEABILITY OF THE SKIN OF APPLES AS MEASURED BY EVAPORATION LOSS¹

K. S. MARKLEY AND CHARLES E. SANDO

Introduction

It has long been known that variations in the skin of fruits influence their keeping quality. This is particularly true in the case of apples (3, 4) where skin condition is recognized as a determining factor in the amount of wilting in storage, susceptibility to scald and other physiological troubles, as a mechanical protection for the underlying tissues, and as a partial regulator in connection with gaseous exchange.

That different varieties of apples differ in skin thickness, amount of waxy coating, and permeability of the skin to gases, especially water vapor, is known in a general way, but little is recorded with respect to their quantitative differences. While the work of CUMMINGS and LOMBARD (1), MAGNESS and DIEHL (4), RIVIÈRE and PICHARD (5), and of HEINICKE (2) is of value in indicating the water loss in relation to the original weight of the whole fruit, it throws but little light on skin permeability because the various workers, with the exception of HEINICKE who worked on a single variety only, failed to calculate their data on an area basis or to account for the loss through the stem and calyx ends.

An opportunity was presented in 1929 to determine under comparable conditions the skin permeability, as measured by evaporation loss, of sixteen varieties of apples, eleven of which were available from two different localities. The fruit used in each case was part of the material assembled for quantitative studies of the cutin and waxy-coating of apples which we have had under way for some time. While the data represent material collected in one season only, it is believed they portray fairly accurately what limits of variation and what varietal differences may be encountered, since all the material was examined under comparable conditions.

In these experiments determinations were made of evaporation losses from normal fruit and from fruit in which the stem and calyx ends were blocked by means of paraffin. The results of these determinations are of interest and importance in that they provide quantitative information upon the varietal differences with respect to the total evaporation loss from the whole apple surface and from the skin exclusive of the stem and calyx openings. Data are given for varieties grown in two dissimilar apple regions,

¹ From the Division of Horticultural Crops and Diseases, Bureau of Plant Industry, in cooperation with the Food Research Division, Bureau of Chemistry and Soils, United States Department of Agriculture, Washington, D. C.

namely, the Wenatchee Valley, Washington, and the Finger Lake region of New York State. From these data it has been possible to calculate for each of the varieties the loss through the skin alone and that by way of the stem and the calyx tube.

Material used

WASHINGTON FRUIT

The Washington fruit was obtained from several commercial orchards in the vicinity of Wenatchee, Washington. The orchards were irrigated during the growing season and were sprayed with lead arsenate in accordance with the usual practice in this district. In addition, Grimes Golden, Delicious, Esopus Spitzenberg, Stayman Winesap, Winesap, Rome Beauty, and Arkansas Black varieties received a one per cent. oil spray during August.

The fruit was harvested one week to ten days earlier than commercial picking time for this district and immediately placed in cold storage at Wenatchee. On November 1 the entire lot of fruit was shipped from Peshastin, Washington, to Philadelphia under initial ice, then by express to Washington, D. C. It arrived November 15 and was immediately placed in cold storage.

NEW YORK FRUIT

The New York State fruit, with the exception of the Arkansas, Winesap, and Stayman Winesap varieties, was obtained from the varietal orchard of the New York State Agricultural Experiment Station at Geneva, through the kindness of RICHARD WELLINGTON and GEORGE H. HOWE. The three excepted varieties were obtained from the varietal orchard of the New York State Agricultural College at Ithaca, through the courtesy of Prof. ARTHUR J. HEINICKE.

The fruit was harvested at the commercial picking date for the particular variety at the place where it was grown, packed at the orchard in lined bushel baskets, and shipped by express to the cold storage house near Washington, D. C. Owing to the abnormal weather conditions prevailing in this region during the season of 1929 (cold, wet spring followed by an unusually dry summer and fall) the New York fruit was, for the most part, somewhat under-developed.

Experimental

About the middle of December the experimental material was withdrawn from cold storage and allowed to stand in the laboratory over night to attain room temperature. The next day each sample was divided into two portions each containing approximately one kilogram of fruit. The diameter of each apple was measured to the nearest millimeter with calipers.

The calyx and the stem openings of the fruit comprising the A-samples were blocked with 45° paraffin, which was introduced while melted. The apples comprising the B-samples remained untreated. The separate lots of fruit were then weighed on a Sartorius precision laboratory balance, arranged on tables, and allowed to remain undisturbed for nine days at an average room temperature of 25.6° C. At the expiration of that time they were again weighed and returned to the tables and left seven more days at the same average room temperature. At the end of this period they were weighed and discarded, since by that time some varieties had undergone considerable shrivelling. All the fruit, both blocked and unblocked, was therefore exposed under identical conditions of humidity and temperature, the latter recorded by means of a thermograph.

Results and discussion

In table I are recorded the total weight of fruit used, the total surface area, the total loss in weight² for the nine and sixteen day periods, and the average daily loss for these periods per 100,000 square millimeters of surface. The data reported under A and B each represent the results of two separate determinations. It should be noted that the surface area has been calculated from the diameter on the assumption that the apples were perfect spheres. This assumption is, of course, only an approximation, but a fairly accurate one for most varieties, since irregularities due to excessive concavity and convexity, particularly in the region of the stem and calyx ends, are more or less compensatory. The varieties are arranged in the decreasing order of the loss through the skin, independent of the loss by way of the stem and calyx tube.

If the figures for the Washington fruit are compared with those for the New York fruit of the same variety it will be seen that except for Grimes Golden and Wagener, the loss per 100,000 square millimeters of skin surface is greater for the New York than for the Washington fruit. Grimes Golden and Wagener apples obtained from Washington were markedly different from those obtained in New York. Before completion of the experiments these varieties suffered from surface scald as well as deep scald and breakdown. Only a single nine day experiment could be made for Wagener because of the severity of the internal breakdown. The considerably larger loss of weight in Washington as compared with New York fruit of these two varieties doubtless is partly the result of the internal breakdown. In

² The evaporation loss determined by the above-described method comprises both transpiration and respiration losses. The latter does not greatly affect the value of the evaporation loss. By employing the figure 35 in milligrams per kilogram-hour as roughly representing an average respiratory rate for apples and computing from this the resultant loss in weight of the fruit it is found that approximately 4 per cent. of the total loss is due to respiration.

TABLE I
EVAPORATION LOSS PER 100,000 MM.² OF APPLE SURFACE

LOCALITY AND VARIETY	SAMPLE	NUMBER OF FRUITS USED	WEIGHT gm.	AREA mm. ²	TOTAL LOSS		AVERAGE DAILY LOSS PER 100,000 MM. ²	
					9 DAYS	16 DAYS	9 DAYS	16 DAYS
					gm.	gm.	gm.	gm.
Washington State fruit	A*	15	2285.78	217,031	105.30	177.17	5.39	5.10
	B	16	2426.33	228,316	123.10	208.09	5.99	5.70
Esopus Spitzenberg	A	15	2332.86	239,315	109.30	186.32	5.30	5.08
	B	15	2355.69	226,120	121.98	208.08	5.99	5.75
Arkansas Black	A	13	2524.57	234,115	83.98	140.15	4.16	3.91
	B	13	2456.74	215,976	83.18	139.45	4.28	4.03
Ben Davis	A	13	2296.68	234,291	83.24	142.40	4.12	3.97
	B	13	2288.40	222,322	93.99	159.39	4.70	4.48
Wagener	A	7	1075.18	113,267	40.55		3.98	
	B	7	1140.43	114,917	44.41		4.29	
Winesap	A	17	2326.76	236,829	83.66	136.71	3.92	3.61
	B	16	2144.43	217,873	81.94	135.02	4.18	3.87
McIntosh	A	15	1936.25	217,911	70.56	121.11	3.60	3.47
	B	15	1821.35	206,105	80.92	140.92	4.36	4.27
Northern Spy	A	15	2080.46	221,725	71.30	129.39	3.57	3.65
	B	7	1000.12	105,756	36.16	64.98	3.80	3.84
Stayman Winesap	A	27	4678.82	442,712	139.38	229.45	3.50	3.24
	B	28	5113.02	475,586	167.75	275.87	3.92	3.62
Delicious	A	15	2385.16	217,238	62.97	105.32	3.22	3.03
	B	15	2470.95	218,429	80.01	135.14	4.07	3.87
York Imperial	A	14	2350.94	232,601	59.05	102.84	2.82	2.76
	B	14	2289.44	229,155	63.99	110.08	3.10	3.00
Rome Beauty	A	12	2388.01	226,249	56.90	94.79	2.79	2.62
	B	12	2480.62	234,809	79.05	131.23	3.74	3.49
Yellow Newtown	A	15	2338.96	240,458	58.61	98.00	2.71	2.55
	B	15	2352.01	242,848	74.32	123.85	3.40	3.19

* A = Stem and calyx ends blocked. B = Normal fruit.

TABLE I (Continued)

LOCALITY AND VARIETY	SAMPLE	NUMBER OF FRUITS USED	WEIGHT gm.	AREA mm. ²	TOTAL LOSS		AVERAGE DAILY LOSS PER 100,000 MM. ²	
					9 DAYS	16 DAYS	9 DAYS	16 DAYS
					gm.	gm.	gm.	gm.
New York State fruit								
Esopus Spitzenberg	A*	20	1848.89	215,203	120.14	207.15	6.20	6.02
	B	20	1713.01	202,784	117.00	200.53	6.41	6.18
Winesap	A	22	1927.31	228,366	116.91	195.13	5.69	5.34
	B	22	1873.53	222,466	133.31	220.74	6.66	6.20
Arkansas	A	12	1993.64	195,147	97.77	164.56	5.57	5.27
	B	12	1976.47	194,257	108.42	181.55	6.20	5.84
Stayman Winesap	A	17	2060.15	225,552	109.78	183.24	5.41	5.08
	B	17	1940.57	214,511	113.18	192.73	5.86	5.62
McIntosh	A	19	1801.94	224,203	98.22		4.87	
	B	19	1783.45	223,308	96.37		4.80	
Northern Spy	A	16	1940.79	208,945	87.47	152.61	4.65	4.56
	B	16	1930.37	224,225	95.73	167.39	4.74	4.67
Baldwin	A	14	1967.45	201,420	83.50	147.12	4.61	4.56
	B	14	1982.86	204,160	100.46	173.91	5.47	5.32
Ben Davis	A	18	1962.70	232,717	91.33	157.81	4.36	4.24
	B	18	1920.82	232,387	95.93	166.36	4.59	4.47
Grimes Golden	A	27	1998.99	248,045	91.25	160.71	4.09	4.05
	B	26	1978.53	245,733	104.09	180.07	4.71	4.57
Rome Beauty	A	19	1901.36	222,450	80.51	135.36	4.02	3.80
	B	19	1957.99	226,999	88.91	147.64	4.35	4.06
Delicious	A	21	1910.79	227,540	79.89	138.76	3.90	3.81
	B	21	2003.89	242,943	95.72	164.61	4.38	4.23
Yellow Newtown	A	33	2046.41	282,134	90.14	154.09	3.55	3.41
	B	33	2107.92	281,594	117.29	201.29	4.63	4.47
Rhode Island Greening	A	17	1937.14	221,951	77.17	119.85		3.37
	B	17	1977.05	223,933	60.52	144.72	3.83	4.04
Wagener	A	22	2019.97	252,820	85.56	106.66	2.66	2.64
	B	22	2044.99	247,662		146.79	3.84	3.70

* A = Stem and calyx ends blocked. B = Normal fruit.

the light of these experiments it would appear that New York fruit possesses, or at least did in 1929, a more permeable skin than Washington fruit of the same variety.

The percentage of water lost by way of the stem and the calyx tube can be calculated from the data given in columns 8 and 9, table I. This loss, expressed as percentage of the total, is given in table II. For the most part, fruit from the two localities show marked differences with respect to

TABLE II

EVAPORATION LOSS THROUGH THE STEM AND CALYX ENDS EXPRESSED AS PERCENTAGE OF THE TOTAL LOSS PER 100,000 MM.² OF APPLE SURFACE

VARIETY	WATER LOST THROUGH STEM AND CALYX ENDS			
	WASHINGTON STATE FRUIT		NEW YORK STATE FRUIT	
	9 DAYS	16 DAYS	9 DAYS	16 DAYS
	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>
Rome Beauty	25.3	24.9	7.6	6.4
Delicious	20.9	21.7	11.0	9.9
Yellow Newtown	20.3	20.1	23.3	23.7
McIntosh	17.4	18.7	0.0	
Ben Davis	12.3	11.4	5.0	5.1
Esopus Spitzenberg	11.5	11.6	3.3	2.6
Stayman Winesap	10.7	10.5	7.7	9.6
Grimes Golden	10.0	10.5	13.2	11.4
York Imperial	9.0	8.0		
Wagener	7.2		30.7	28.6
Winesap	6.2	6.7	14.6	13.9
Northern Spy	6.0	4.9	1.9	2.4
Arkansas Black	2.8	3.0		
Baldwin			15.7	14.3
Rhode Island Greening			15.4	16.6
Arkansas			10.2	9.8

stem end and calyx end losses. Except for Grimes Golden, Yellow Newtown, Winesap, and Wagener, which are higher in the Eastern fruit, the Washington fruit showed the higher, often a considerably higher loss by way of the stem and the calyx tube than did the New York fruit. Because of the larger size of the Washington fruit it might be expected that their calyx end loss would be larger than that of the underdeveloped and consequently smaller New York fruit. This is in accord with the observations of YOUNG (6) to the effect that the calyx cup or eye of the apple is influenced by the development of the fruit. According to these observations the calyx tube of large fruit is apt to be large, so that the lobes are separated, resulting in an open or partly open calyx, whereas in small or poorly developed apples the calyx is usually small.

Summary

These studies show that in 1929 New York apples had a higher water loss per 100,000 square millimeters of surface than Washington fruit, with the exception of Grimes Golden and Wagener, both of which were abnormal. It may be concluded from these results that the New York fruit had more permeable skins than Washington fruit.

The stem and calyx end losses varied from zero to 31 per cent. in different varieties, and the variation was almost as great for the same varieties in different localities as for different varieties in the same localities.

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RESPIRATION STUDIES OF STRAWBERRIES¹

E. L. OVERHOLSER, MAX B. HARDY, AND H. D. LOCKLIN

In the study of promising new varieties of strawberries, it is desirable to employ some measurement that will give satisfactory evidence of their keeping quality when compared with standard varieties in a district. To this end, HARDY and LOCKLIN (unpublished) have employed a test by means of which the firmness of the fruit is expressed in grams of pressure necessary to force a one-eighth inch plunger through the flesh to a depth of three-sixteenths of an inch. It was noted, however, that frequently the firmness of the flesh of varieties was not consistently correlated with casual observations as to the keeping quality at laboratory temperatures. This indicated that varieties having the most firm flesh did not necessarily possess the lowest rate of metabolism.

Objects of experiments

Experiments were therefore undertaken to determine the correlation of the rate of metabolism or respiration intensity as measured by the production of carbon dioxide, with certain factors as follows: (1) Firmness of tissue; (2) maturity; (3) time of season harvested; (4) succession of respiration interval; (5) duration of respiration period and the percentages of carbon dioxide and oxygen in the respiration chamber; and (6) variety. Incidentally the relation of the respiration ratio and of the specific gravity of strawberries to certain factors was observed.

Methods employed

Respiratory intensity was used as a measure of rate of metabolism in the work reported here. One-quart fruit jars sealed with rubber corks fitted with stop-cocks were used as respiration chambers. The temperature in these chambers averaged about 20° during the investigation with variations of $\pm 2^\circ$ C. The intensity of respiration was determined by methods of BENNETT and BARTHOLOMEW (1). The respiration interval averaged about 16 hours, and the jars were opened and the berries aerated for about six hours before another respiration interval was begun. The lots of strawberries employed in these studies were grown upon a medium heavy clay loam on the grounds of the Western Washington Experiment Station, Puyallup, Washington. The strawberries were harvested for each determination at an immature stage (10 to 15 per cent. colored) and at a mature

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stage (100 per cent. colored). The fruit was brought directly from the field to the laboratory and utilized at once for the studies.

Results

RELATION OF FIRMNESS OF TISSUE TO RESPIRATION INTENSITY

Using the method of HARDY and LOCKLIN the firmness of the flesh of the strawberry varieties was determined. From one to four penetrations per berry of 10 to 15 berries were averaged.

The respiration intensity of certain varieties having relatively firm tissue was compared with other varieties having relatively soft tissue. For these determinations fruits were used that were well matured with 100 per cent. color and also immature fruits with only from 10 to 35 per cent. color. The average duration of each respiration period was about 16 hours. The data obtained with the immature berries of different degrees of firmness of flesh are presented in table I.

The data in table I indicate that firmness of flesh of immature berries is not correlated with low respiration intensity. Each of three firm varieties, harvested in an immature stage of ripeness had a higher respiration intensity than did three soft fleshed varieties with the exception of the Klondike. This was true not only of the average as shown in table I, but

TABLE I

RELATION OF FIRMNESS OF TISSUE OF VARIETIES OF IMMATURE STRAWBERRIES TO RESPIRATION INTENSITY

VARIETY	DATES HARVESTED	COLOR	FIRMNESS OF TISSUE	NUMBER OF RESPI- RATION PERIODS DETER- MINED	CO ₂ PRO- DUCED PER KILO PER HOUR	RESPIRA- TION RATIO CO ₂ /O ₂
		<i>per cent.</i>	<i>gm.</i>		<i>mg.</i>	
U. S. D. A. 632 .	June 14, 20	12	511	5	89.68	0.836
U. S. D. A. 819 .	" 14, 17	27	548	6	73.82	0.842
Ettersburg 121 .	" 20	20	421	3	88.24	0.852
Average for firm fleshed varie- ties			493		83.91	0.843
U. S. D. A. 652	June 14, 17, 20	10	284	9	45.20	0.831
U. S. D. A. 545 .	" 14, 17	10	333	6	65.65	0.844
Klondike	" 20	35	276	3	88.48	0.913
Average for soft fleshed varie- ties			308		66.44	0.863

also of each individual determination. The Klondike, however, was more mature than any of the other immaturesly harvested varieties in that the berries were over one-third colored. This greater maturity in the light of data subsequently presented apparently increased the intensity of respiration. If this be true, which is not unlikely, the average value for this group would be still lower.

Similar determinations were made with berries harvested in the mature stage. These data are shown in table II.

TABLE II

RELATION OF FIRMNESS OF TISSUE OF VARIETIES OF MATURE (100 PER CENT. COLORED) STRAWBERRIES TO RESPIRATION INTENSITY

VARIETY	DATES HARVESTED	FIRMNESS OF TISSUE	NUMBER OF RESPIRATION PERIODS DETERMINED	CO ₂ PRODUCED PER KILO PER HOUR	RESPIRATION RATIO CO ₂ /O ₂
		<i>gm.</i>		<i>mg.</i>	
U. S. D. A. 632	June 14, 17, 20	357	9	127.89	0.906
Ettersburg 121	" 20	372	3	98.74	0.901
U. S. D. A. 527	" 14	321	3	95.21	0.810
U. S. D. A. 819	" 14, 17	343	6	83.11	0.887
Average for firm fleshed varieties		348		101.27	0.878
U. S. D. A. 652	June 14, 17, 20	214	9	75.73	0.879
U. S. D. A. 177	" 14	261	3	84.15	0.925
U. S. D. A. 545	" 14, 17	239	6	64.42	0.896
Klondike ..	" 20	287	3	95.69	0.955
Average for soft fleshed varieties		250		79.99	0.914

The data from the strawberries harvested when mature also indicated that firmness of flesh did not necessarily mean a low respiration intensity. Comparison of the respiration ratios in tables I and II indicated that the more mature berries had a higher respiration ratio (carbon dioxide evolved divided by the oxygen absorbed) than did the immature berries (see also table III) and that the firm fleshed varieties had a higher respiration ratio than the soft fleshed sorts.

RELATION OF MATURITY TO RESPIRATION INTENSITY

Five varieties of strawberries were harvested in an immature stage of development (10 to 25 per cent. colored) and their respiration intensity

compared with that of the same varieties harvested in a well matured stage (100 per cent. colored). The data are shown in table III.

TABLE III
RELATION OF MATURITY OF STRAWBERRIES TO RESPIRATION INTENSITY

VARIETY	MATURITY	FIRMNESS OF TISSUE	NUMBER OF RESPIRATION PERIODS DE- TERMINED	CO ₂ PRO- DUCED PER KILO HOUR	RESPIRATION RATIO CO ₂ /O ₂
		<i>gm.</i>		<i>mg.</i>	
U. S. D. A. 632	Immature	512	6	89.68	0.836
U. S. D. A. 652....	Immature	284	9	45.20	0.831
U. S. D. A. 261 ...	Immature	346	6	90.70	0.872
U. S. D. A. 545	Immature	353	6	65.65	0.844
U. S. D. A. 819 . .	Immature	548	6	73.82	0.842
Average for imma- ture berries . .		409	33	73.01	0.845
U. S. D. A. 632 . .	Well matured	355	9	117.89	0.906
U. S. D. A. 652	Well matured	214	9	75.73	0.879
U. S. D. A. 261	Well matured	234	6	112.50	0.921
U. S. D. A. 545	Well matured	239	6	69.42	.0913
U. S. D. A. 819 .	Well matured	327	6	83.11	0.887
Average for mature berries . . .		274	36	91.73	0.905

The data in table III show that as the strawberries advance from the immature stage, where the fruit is only 10 to 25 per cent. colored, to the well matured stage with 100 per cent. color, the rate of respiration increases. The average increase is nearly 50 per cent., with the exception of the variety U. S. D. A. 545, where the increase is relatively small. As indicated by a comparison of the data in tables I and II, the data in table III also show that the respiration ratio of mature berries was nearer unity than that of the immature berries. This indicates, as would be expected, that the material respired, by the mature berries is not the same as that in the immature fruit.

RELATION OF TIME OF SEASON HARVESTED TO RESPIRATION INTENSITY

It is reported by growers that strawberries picked at the same stage of maturity late in the fruiting period for the variety do not keep nor ship as well as berries harvested earlier in the season. Data pertaining to the relation of time of the season the strawberries were harvested, (the maturity being the same) to respiration intensity are shown in table IV.

The data in table IV show that as the season advances strawberries even though picked at comparable stages of maturity exhibit higher respiration

TABLE IV

RELATION OF TIME OF SEASON HARVESTED TO RESPIRATION INTENSITY OF STRAWBERRIES OF COMPARABLE MATURITY

VARIETY	DATES HARVESTED	MATURITY	COLOR	FIRMNESS OF TISSUE	CO ₂ PRODUCED PER KILO PER HOUR	RESPIRATION RATIO CO ₂ /O ₂
			<i>per cent.</i>	<i>gm.</i>	<i>mg.</i>	
U. S. D. A. 652	June 14	Immature	10	297	36.09	0.827
	June 17	Immature	10	293	47.67	0.838
	June 14	Well matured	100	204	59.30	0.880
	June 17	Well matured	100	208	79.47	0.952
U. S. D. A. 632	June 14	Immature	10	562	80.54	0.813
	June 20	Immature	15	461	98.82	0.859
	June 14	Well matured	100	317	110.78	0.907
	June 17	Well matured	100	365	126.63	0.926
U. S. D. A. 545	June 14	Immature	10	347	60.69	0.797
	June 17	Immature	10	359	70.61	0.890
U. S. D. A. 261	June 14	Immature	15	364	89.03	0.868
	June 17	Immature	15	327	94.84	0.897
	June 14	Well matured	100	239	102.34	0.927
	June 17	Well matured	100	228	122.34	0.974

intensities. This was true of berries harvested in an immature stage as well as berries harvested when fully mature. The average respiration intensity of the immature and mature strawberries harvested early in the season was 78.7 mg. of carbon dioxide per kilogram of fruit in contrast with 90.4 for fruit harvested later in the season. The data are all the more significant when it is pointed out that the temperatures in the field averaged two degrees higher on June 14 than on June 17.

RELATION OF SUCCESSION OF RESPIRATION INTERVAL TO RESPIRATION INTENSITY

The greater respiration intensity of berries which accompanied their advance in maturity was also indicated by the fact that generally the respiration rate tended to increase with each succeeding respiration period of fifteen to eighteen hours. This increase was more marked with the maturely harvested fruit than with the fruit harvested when immature, as shown by the average of eight varieties in table V.

RELATION OF DURATION OF RESPIRATION PERIOD AND THE PERCENTAGES OF CARBON DIOXIDE AND OXYGEN IN THE RESPIRATION CHAMBERS

Attempts were made to keep the respiration intervals fairly uniform and to avoid the accumulation of excess carbon dioxide and marked reduc-

TABLE V

RELATION OF SUCCESSION OF RESPIRATION INTERVAL TO RESPIRATION INTENSITY

ORDER OF RESPIRATION INTERVAL	NUMBER OF DETERMINA- TIONS	DURATION OF RESPIRATION INTERVAL	MATURITY OF STRAWBERRIES	CO ₂ PRODUCED PER KILO PER HOUR
		<i>average hrs.</i>		<i>mg.</i>
First	15	18.24	Immature	72.56
Second	15	15.03	Immature	79.80
Third	15	15.25	Immature	87.17
First	20	18.16	Mature	80.33
Second	20	15.22	Mature	90.59
Third	20	15.45	Mature	102.69

tion of the oxygen content of the air of the respiration chambers. An increase in the carbon dioxide generally has a depressing effect upon respiration of some tissues, which is more marked if the temperature is low, as shown by KIDD (3) with germinating seeds, and by KIDD, WEST and KIDD (4) with apples, and TROUT (5) with pears. The effect is more marked at temperatures lower than those employed here for the respiration studies of strawberries. An appreciable reduction in the oxygen content may, however, have little or no effect upon respiration as measured by carbon dioxide production. In our experiments the percentage of oxygen was reduced about the same as the percentage of carbon dioxide was increased. Generally, however, slightly more oxygen was consumed than carbon dioxide was evolved. The data show the average milligrams of carbon dioxide produced per kilogram of fruit during the respiration interval when the carbon dioxide content of the atmosphere in the respiration chambers became highest, in comparison with the production by the same lots during the respiration interval when the carbon dioxide content remained lowest. The data showing the relation of these factors to the respiration intensity are shown in table VI.

TABLE VI

RELATION OF DURATION OF RESPIRATION PERIOD AND PERCENTAGE OF CARBON DIOXIDE AND OXYGEN TO RESPIRATION INTENSITY

AVERAGE DURATION OF RESPIRATION INTERVAL	MATURITY OF FRUIT	NUMBER OF DETERMINA- TIONS	AVERAGE PER CENT. OF CO ₂ IN RESPIRA- TION CHAMBER	CO ₂ PRODUCED PER KILO PER HOUR
<i>hr.</i>			<i>per cent.</i>	<i>mg.</i>
14.56	Immature	17	7.86	79.07
16.97	Immature	17	10.41	82.34
15.20	Mature	19	9.36	87.55
17.94	Mature	19	12.50	98.71

TABLE VII
RELATION OF VARIETY OF STRAWBERRIES TO RESPIRATION INTENSITY

VARIETY	MATURITY	COLOR	FIRMNESS OF TISSUE	NUMBER OF PICKINGS	NUMBER RESPIRATION PERIODS DE- TERMINED	CO ₂ PRODUCED PER KILO PER HOUR	RESPIRATION RATIO $\frac{\text{CO}_2}{\text{O}_2}$	SPECIFIC GRAVITY
U. S. D. A. 652	Immature	<i>per cent.</i> 10	286	<i>Ave.</i> 3	9	<i>mg.</i> 42.20	0.831	0.904
U. S. D. A. 652	Mature		224		9	75.73	0.879	0.890
U. S. D. A. 632	Immature	10	512	3	9	89.68	0.836	0.951
U. S. D. A. 632	Mature	100	341	3	9	118.71	0.917	0.982
U. S. D. A. 261	Immature	15	346	2	6	91.99	0.883	0.906
U. S. D. A. 261	Mature	100	234	2	6	112.34	0.921	0.958
U. S. D. A. 545	Immature	10	353	2	6	65.65	0.844	0.929
U. S. D. A. 545	Mature	95	239	2	6	69.42	0.913	0.896
U. S. D. A. 819	Immature	27	548	2	6	73.82	0.842	0.913
U. S. D. A. 819	Mature	100	327	2	6	83.11	0.887	0.942
Klondike	Immature	35	287	1	3	88.48	0.913	0.937
Klondike	Mature	100	257	1	3	95.69	0.955	0.937

The data show that there was no depressing effect of the longer respiration intervals or of slightly greater increases of carbon dioxide contents of the respiration chambers. In fact the respiration intensities of both the mature and immature fruits were slightly greater during the longer intervals and with the higher carbon dioxide content. This was consistently true of the individual determinations as well as the average given in table VI. Furthermore, the average respiration ratio was below unity, indicating that there was an absence of intramolecular respiration.

RELATION OF VARIETY TO RESPIRATION INTENSITY

The data in table VII show that there are consistent differences in respiration intensity between varieties. These differences again were not necessarily correlated with keeping quality at room temperature. For example, while some firm varieties respired more rapidly than did medium firm varieties the former may have been sufficiently more firm, so that notwithstanding their greater respiration intensity, they were still less soft and in better market condition than were the medium firm, after a given interval of time.

RESPIRATION RATIO AND SPECIFIC GRAVITY OF STRAWBERRIES

The average respiration ratio of mature strawberries, 100 per cent. colored and softening, was somewhat higher than that of immature berries, 10 per cent. colored and firm. In each case, however, it was below unity, being in the former 0.909 and in the latter 0.870. GERHART (2) reported that the approximate value of the respiration ratio of ripe strawberries was 1.2 as determined by the Krajnik apparatus. This would indicate that more carbon dioxide was evolved than oxygen was absorbed, while with the data presented in table VIII, the reverse appears to be true.

TABLE VIII

AVERAGE RESPIRATION RATIO AND SPECIFIC GRAVITY OF STRAWBERRIES

MATURITY	NUMBER OF VARIETIES	RESPIRATION RATIO $\frac{\text{CO}_2}{\text{O}_2}$	SPECIFIC GRAVITY	NUMBER OF DETERMINATIONS	
				RESPIRATION	SPECIFIC GRAVITY
Immature ..	12	0.870	0.860	48	17
Mature	11	0.909	0.923	57	19

The data in table VIII indicate that with the varieties employed the specific gravity of the mature fruit averaged slightly higher than for the immature berries.

Summary

1. With the strawberry varieties tested greater firmness of flesh apparently was not directly correlated with a low respiration intensity, either with immature or mature fruits.
2. With the varieties employed fruits of the firm-fleshed sorts had a higher respiration ratio than fruits of the soft-fleshed sorts.
3. The respiration intensity increased with the maturity of the fruit.
4. Both immature and mature fruits when picked at intervals in comparable stage of maturity showed a higher respiration intensity and a higher respiration ratio as the picking season advanced.
5. The respiration intensity of any one lot of mature or immature fruits increased with each succeeding respiration interval, the increase being more marked with the mature lots of fruit.
6. No apparent depression of the respiration intensity followed the increased carbon dioxide contents attained in the respiration chambers resulting from a lengthening of the respiration period.
7. There are consistent differences between varieties in their respiration intensities which were not, however, directly correlated with keeping quality, since the latter was affected also by the initial firmness of the berries.
8. Greater maturity of fruit was correlated with a higher respiration ratio, although no single value ever attained unity.
9. The specific gravity of the mature fruits of the varieties employed average slightly higher than that of the immature fruits.

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EFFECT OF MANGANESE, COPPER AND ZINC ON THE GROWTH OF YEAST¹

J. S. MCHARGUE AND R. K. CALFEE

(WITH THREE FIGURES)

The fermentation of sugars by yeast has received much scientific investigation. The more common mineral elements required in relatively large amounts for the growth of yeasts have been definitely established but the influence of some of the less common elements in the metabolism of yeasts is not so well known. Manganese, copper and zinc can be found in small amounts in the ash of most plant material. Because yeasts depend upon the products of other plants for a portion of their nutrients, they have access to these metals.

KAYSER and MARCHAND (1) state that the addition of from 1 to 1.5 gm. of manganese sulphate per liter increased the yield of alcohol 3 per cent. in some cases. The amounts of glycerol and volatile acids were also increased. They obtained especially good results with the nitrate.

KOSTYTSCHEW and FREY (2) found that zinc chloride added at the rate of 0.4 gm. to 2.6 gm. in 100 cc., caused the production of acetaldehyde in dry but not in living yeast fermentation. It also resulted in an increased production of carbon dioxide as compared with the alcohol, and in a large part of the fermented sugar being converted into unknown products. The larger part of the sugar remained unfermented in the presence of zinc chloride whereas practically all was fermented in controls without zinc.

SCHWEIZER (3) found that fermentation was decreased in copper vessels as compared with glass. He investigated the effect of cuprous oxide and found it decreased growth in amounts greater than 0.0001 gm. for 0.5 gm. of yeast, and that 0.014 gm. inhibited fermentation. Cupric oxide did not affect fermentation.

The chief difficulty in investigating the influence of manganese, copper and zinc on the growth of yeasts was in obtaining a medium suited to their growth that was free from traces of these metals. The medium used in this investigation was an aqueous solution containing ammonium sulphate 0.5 per cent., potassium dihydrogen phosphate 0.2 per cent., potassium sulphate 0.4 per cent., magnesium sulphate 0.1 per cent., calcium sulphate 0.05 per cent., ferrous chloride 0.001 per cent., and sucrose 5.0 per cent.

¹ Contribution from the Department of Chemistry of the Kentucky Agricultural Experiment Station.

The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

The inorganic salts used were tested chemically and were known to be free from copper, manganese and zinc. Sucrose could not be obtained entirely free from zinc, but less than 0.2 part per billion was contained in the medium, from this source. The water required was distilled through a quartz tube. This medium was used as the control medium.

Pure cultures of *Saccharomyces cerevisiae* were prepared and transferred on the control medium six times to remove manganese, copper and zinc from the cells before a stock culture was accepted for inoculation of experimental cultures. Counts were then made microscopically and a suspension in control medium was standardized so that one cubic centimeter contained one thousand cells. One hundred cells (0.1 cc.) were used for inoculation of most cultures.

To determine the effect of different concentrations of manganese a series of ten cultures of 100 cc. each, to which different quantities of manganese sulphate had been added, was started. Manganese was added in such proportions that the first flask contained 10 parts per million, the second 20 ppm., the third 30 ppm., etc., covering the range between 0 and 100 ppm. manganese. Duplicate cultures and two controls were prepared. Other factors such as the time and temperature of incubation and sterilization, and number of cells used for inoculation, were the same in all experimental cultures. After six days' incubation, the cultures were filtered and the yeast cells washed with cold distilled water in excess of the amount found necessary to remove sulphates. The same volume of water was used on each culture. The washed cells were dried to constant weight at 100° C. and weighed as the quantity produced in 100 cc. of medium in six days. Comparison of the weights obtained showed that the smaller quantities of manganese stimulated growth, as determined by weight, that large quantities were toxic, resulting in reduced growth or death, and that a concentration of 10 ppm. resulted in the largest weight obtained. A second series of 10 cultures covering the range between 5 and 15 ppm. manganese fixed the optimum concentration of this element at 10 ppm.

By using similar series of cultures containing copper sulphate, and zinc sulphate, the optimum concentrations were found to be 7.5 ppm. for copper and 10 ppm. for zinc. These values were used in preparing media for the following cultures.

To ascertain the effect of these metals on growth as determined by total weight, cultures of 250 cc. in liter flasks were prepared as follows: 1, control; 2, + manganese; 3, + copper; 4, + zinc; 5, + manganese + copper; 6, + manganese + zinc; 7, + copper + zinc; 8, + manganese + copper + zinc. These cultures, with duplicates, were incubated for ten days at 28° C. and the growth produced was weighed as previously described. Fig. 1 shows

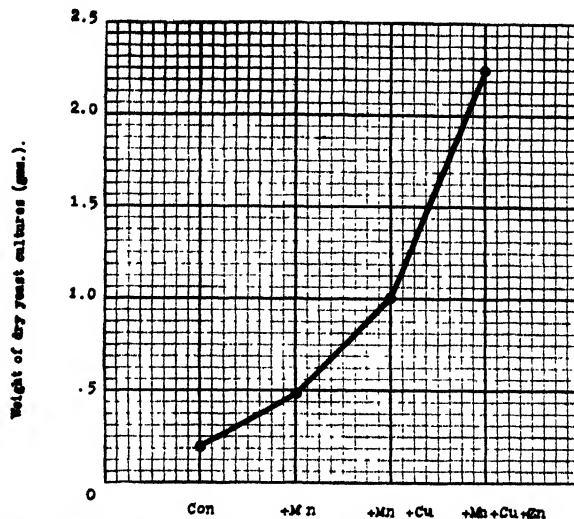


FIG. 1. Addition of Mn, Cu, and Zn to the control medium—effect on weight of cells.

the results obtained. Copper produced the greatest weight of any element when present without the others. The values for zinc were slightly greater than those for manganese. Combinations of two metals produced greater weights than did either one alone. The largest weight obtained resulted from the inclusion of manganese, copper and zinc in the medium. The relations to the control are shown in table I.

To determine the effect on growth as indicated by rate of cell division, the number of cells per cc. was counted daily, microscopically, in a gradu-

TABLE I
PRODUCTION OF YEAST CELLS (AVERAGE OF 3 CULTURES)

CULTURE	TOTAL WEIGHT	PER CENT. OF CONTROL
	<i>gm.</i>	<i>per cent.</i>
Control	0.2064	100
+ Mn	0.4934	239
+ Cu	0.5915	286
+ Zn	0.5364	260
+ Mn + Cu	1.0138	491
+ Mn + Zn	1.9711	950
+ Cu + Zn	1.1461	555
+ Mn + Cu + Zn	2.2506	1090

ated cell slide, in cultures of 250 cc. in 500-cc. flasks. The initial inoculation was 2,500 living cells. Counts were discontinued at the end of six days, because of bacterial contamination in some flasks. Fig. 2 shows the

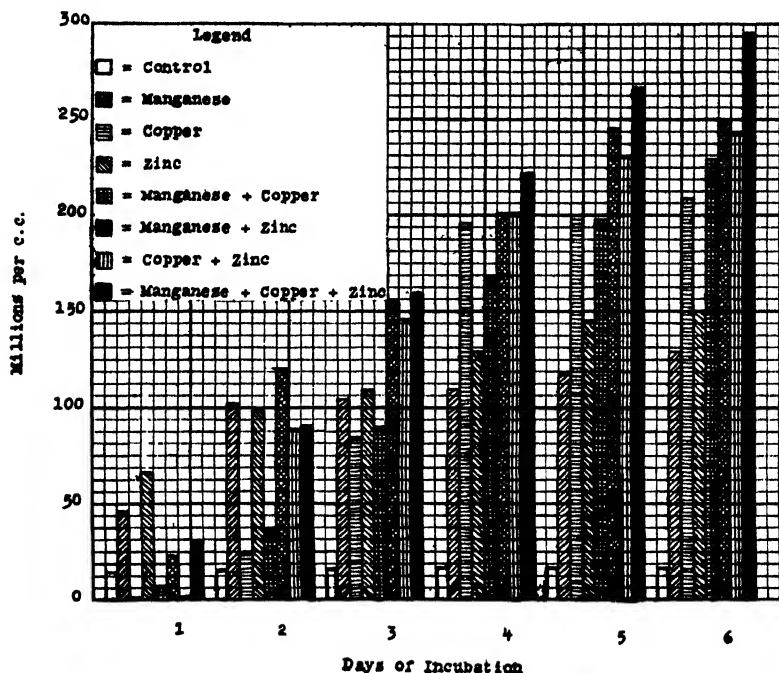


FIG. 2. Addition of Mn, Cu, and Zn to control medium—effect on number of cells.

effect of manganese, copper, zinc, and their combinations on the rate of cell division. Copper inhibited reproduction at the start but, as incubation proceeded, increased it more than did either manganese or zinc. Manganese and zinc, separately, produced immediate increases in the rate of cell division as compared with the control.

The cells in media containing copper were small, the average of the mature cells being 6.6×7.7 microns, slightly less than the control measurements. Manganese and zinc, separately, increased the size of cells, the average being 8.25×9 microns for each. The fact that cultures containing copper resulted in a greater weight of yeast than did cultures containing manganese or zinc was due to the presence of a larger number of smaller cells. The increase in weight of yeast in the cultures containing manganese and zinc over that in the cultures containing copper and zinc, or copper and manganese, was caused by a larger size of cell, the number of cells being nearly the same. With manganese, copper and zinc together the cells were but slightly smaller than they were in the presence of man-

ganese and zinc, and as the rate of cell division was much greater, the largest weight obtained resulted from this culture.

The effect of manganese, copper and zinc on the production of carbon dioxide by yeast was determined by direct weighing of the absorbed gas. Cultures of 250 cc. each, in liter flasks, containing manganese, copper, zinc, and all combinations of these, in optimum concentrations, and controls, were prepared. The flasks were sealed with rubber stoppers fitted with two glass tubes, one of which extended below the surface of the medium. These tubes were connected with soda-lime and calcium chloride tubes as for carbon determination. Air entered the long tube through sterile cotton and a soda-lime tube, and bubbled through the culture. Gases left the culture flask through calcium chloride tube, a weighed soda-lime tube, a second calcium chloride tube, and the aspirator. The weights of carbon dioxide absorbed by the soda-lime were recorded daily. The number of bubbles per minute that passed through the culture was regulated by a clamp on the rubber tube leading to the aspirator, and was kept constant for all cultures. Cultures were aerated continuously except while weighings were being made, and were kept at a constant temperature of 28° C. for 12 days (average of three series).

Carbon dioxide was produced irregularly in the same culture, and at different rates in media containing different treatments. The period over which it was produced also was influenced by the treatments. The presence of bacteria (acetobacter) was found to increase the carbon dioxide value; therefore all contaminated cultures were discarded.

The presence of manganese, copper or zinc in the medium increased the rate of production and the total amount of carbon dioxide produced, as shown in table II.

TABLE II
PRODUCTION OF CARBON DIOXIDE, AEROBIC FERMENTATION
(AVERAGE OF 3 SERIES)

CULTURE	WEIGHT CO ₂ <i>gm.</i>	PER CENT. OF CONTROL
		<i>per cent.</i>
Control	0.2770	100
+ Mn	0.6191	224
+ Cu	0.8474	306
+ Zn	0.8102	293
+ Mn + Cu	1.0154	367
+ Mn + Zn	1.1384	410
+ Cu + Zn	1.1230	405
+ Mn + Cu + Zn	1.1657	421

Copper produced a greater effect than did manganese or zinc. Manganese and zinc shortened the period of production as compared with the control while copper extended it. The addition of either manganese, copper or zinc to a culture not containing it resulted in an increased production of carbon dioxide (fig. 3). The production of carbon dioxide varied roughly with the number of cells present in the culture.

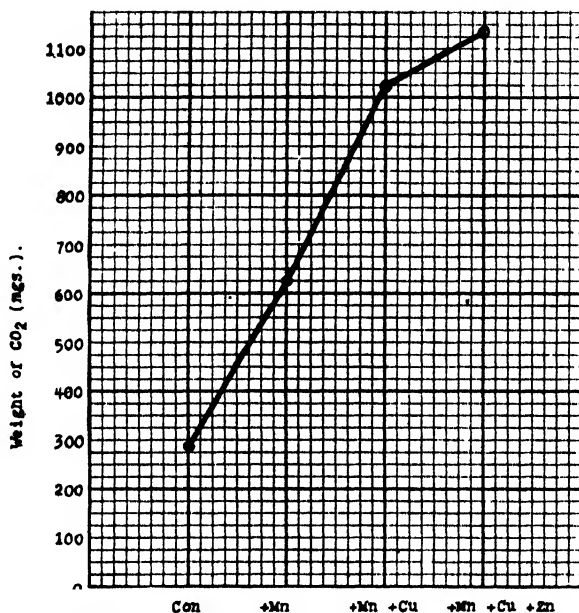


FIG. 3. Addition of Mn, Cu, and Zn to control medium—effect on production of CO₂.

In comparison with the theoretical yield, the quantities of carbon dioxide produced by all experimental cultures were low. Analysis of the medium showed inverted sugars to be absent in all treated cultures, and to be present only in traces in the control cultures, so the sucrose was evidently fermented as rapidly as it was inverted. To determine the effect that oxygen passing through the cultures might have had on the production or action of invertase, carbon dioxide was determined on a series of cultures under anaerobic conditions, imposed by using nitrogen gas to displace the carbon dioxide. These cultures, in comparison with similar aerobic cultures showed a slight increase in the average daily production of carbon dioxide. The period of active fermentation was from four to seven days longer under anaerobic conditions, the extension being greatest for the control cultures. The result was an appreciable increase in the total production of carbon dioxide. A more marked increase occurred in the treated

cultures, with the exception of the ones receiving only copper, than occurred in the controls. Copper, at this concentration, was toxic under anaerobic conditions and, when present in cultures, retarded the production of carbon dioxide except in the cultures treated with manganese and zinc. Table III shows the total amounts of carbon dioxide evolved under anaerobic fermentation of sucrose. The retarding effect of oxygen on the evolution of carbon dioxide is evident upon comparing table II with table III.

TABLE III
PRODUCTION OF CARBON DIOXIDE, ANAEROBIC FERMENTATION

CULTURE	WEIGHT CO ₂	PER CENT. OF CONTROL
	<i>gm.</i>	<i>per cent.</i>
Control	0.3829	100.0
+ Mn	1.3552	353.9
+ Cu
+ Zn	1.5604	407.5
+ Mn + Cu	1.4726	384.5
+ Mn + Zn	3.0827	805.09
+ Cu + Zn	1.4679	383.3
+ Cu + Mn + Zn	3.6579	955.2

Invert sugar could not be detected in the medium of any culture of the anaerobic series. Sucrose was found to be present in all cultures in such quantities that when calculated to carbon dioxide, it compared favorably with the difference between the theoretical yield and the quantity evolved during fermentation, verifying the carbon dioxide determinations. Sucrose was fermented under anaerobic conditions as rapidly as it was inverted.

The weights of yeast obtained under anaerobic fermentation were slightly less than in the corresponding aerobic cultures. Atmospheric oxygen did not retard the growth of yeast cells, but lowered their efficiency in fermenting sucrose, presumably by inhibiting the production, or interfering with the catalytic action of the enzymes, especially invertase.

To ascertain the effect of the absence of manganese, copper and zinc upon zymase, anaerobic controls, each containing dextrose in an equivalent quantity to the sugar in the sucrose cultures were investigated. Dextrose could not be obtained entirely free from copper and zinc, but the quantities of these metals in the cultures were considerably below the optimum. Fermentation started immediately, carbon dioxide being evolved at a relatively uniform rate, averaging 0.045 grams a day, and decreasing slowly

as fermentation slackened. The total production of carbon dioxide in dextrose control cultures averaged 0.8279 gm. This quantity was greater than that produced by sucrose controls, but less than that of treated sucrose cultures. Evidently the structure of the sugar was not entirely responsible for the low production of carbon dioxide, normal growth of the yeast and production of zymase not occurring in the absence of manganese, copper and zinc.

Summary

1. The sulphate of manganese, copper and zinc, in small quantities, increased the dry weight of yeast produced.
2. Excessive quantities of manganese, copper and zinc salts were toxic, resulting in decreased growth or death of the cells.
3. Copper stimulated cell division and resulted in cells of smaller size than in the controls.
4. Manganese, copper and zinc stimulated production of carbon dioxide, copper being more effective than manganese or zinc.
5. Carbon dioxide was produced in larger quantities under anaerobic fermentation than under aerobic conditions.
6. Control cultures containing dextrose gave but slightly more carbon dioxide than did the sucrose controls.

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INFLUENCE OF DRY SOIL ON ROOT EXTENSION

A. H. HENDRICKSON AND F. J. VEIHMEYER

(WITH SEVEN FIGURES)

Previous work at this Station (2) shows that the percentages for the relative wetness of soils expressed as ratios of soil-moisture contents to their respective moisture equivalents may be used to show the development of roots, and the results of adequate moisture samples, taken at proper times, indicate with a fair degree of accuracy the presence or absence of roots growing in the soils tested. It was further concluded, "that if the soil is wet at the beginning of the growing season to the full depth to which roots of plants would normally penetrate, subsequent additions of water by rain or irrigation, unless adverse conditions for growth are brought about thereby, can have but little influence on the extent of the root system developed."

In connection with the studies from which these findings were drawn, the question arose as to whether roots would elongate into dry soil. SHANTZ (6) believes that some drought resistant plants do have the ability to push their roots through dry soil, but that ordinary field crops do not. MAGISTAD and BREAZEALE (5), also, believe that some plants will send their roots through dry soil and BREAZEALE (1) thinks that roots will elongate into dry soil because of the "build up" of the moisture content of the dry soil by water transported from the roots in moist soil.

LIVINGSTON's work (3) at Tucson, Arizona, shows that the roots of seedlings penetrated downward when the soil was wet from the frequent showers of the rainy season, but there is no evidence in his report to show that the roots of these plants had the ability to traverse dry soils.

In the work previously mentioned (2), it was reasoned, largely from theoretical considerations, that roots do not penetrate dry soils. Since the publication of the previous report some simple experiments have been carried out to ascertain the influence of dry soil on root extension.

Penetration of roots through wax seals into wet and dry soil

During the summer of 1929 a number of large cans with a capacity of about ten quarts, holding about 15 kilograms of soil, were filled with soil in somewhat the same manner as described by WEAVER, JEAN, and CRIST (8). The bottom half of each can was filled with soil, the moisture content of which was brought up to a certain percentage by spraying the water on the mass of dry soil while constantly turning the latter until sufficient water

had been added to bring about the desired moisture content. The soil was packed in the bottom half of the container and was covered with a layer of wax composed of approximately 80 per cent. paraffin, 10 per cent. petrolatum, and 10 per cent. beeswax. The soil used was a Yolo clay from Davis, California, with a moisture equivalent of about 30 per cent. and a residual moisture content at permanent wilting of plants, or, as we designate it, a permanent wilting percentage of about 14 per cent. The cans were half filled with soil containing 5, 10, 14, 15, 20, and 30 per cent. of moisture. Four cans were used for each trial. The upper half of each can above the wax seal was then filled with dry soil which was tamped in place, and sufficient water was added to it to raise the moisture content to the moisture equivalent.

Sunflower seeds were planted in the cans, and, after the plants had emerged, the surface of the soil was covered with a layer of tin foil to keep down evaporation. Water was added to the surface of the soil from time to time to maintain a supply of soil moisture readily available in the upper half of the cans. After the plants had grown to a height of about 18 inches, the cans were cut with tinner's snips and the cut portion bent down to expose the soil. The soil in the bottom half of the cans below the wax seal was carefully examined for roots.

Roots were present in those portions of the cans that contained soil that was above the permanent wilting percentage. In a few of the cans with soils drier than the permanent wilting percentage, the wax seal between the two layers had not remained intact and some of the water from the irrigations applied to the soil in the upper half had seeped into the lower half. In those cases where it was apparent that the soil had been moistened by the downward movement of water through the wax seal, roots were found in the lower portion. No roots, however, were found in the lower layer when the wax seal remained intact and the soil-moisture content remained below the permanent wilting percentage. In a number of cases it was noted that the roots actually penetrated through the wax seal, but did not grow more than a few millimeters into the dry soil or soil which contained less moisture than the permanent wilting percentage. These experiments seemed to substantiate our belief that roots would not be pushed into dry soil; but being somewhat indecisive, because water leaked through the wax seals in some cases, we decided to make more reliable tests.

In a subsequent experiment a series of wooden troughs, 180 cm. long, 15 cm. deep, and 15 cm. wide, lined with galvanized iron was used. The troughs were divided into three equal sections by partitions of 6 mm. mesh hardware cloth. A wax of the same composition as that previously mentioned was heated and brushed onto the wire cloth which was held in place

in the troughs by means of cleats. Several layers of wax were brushed on, allowing each to cool before application of the next layer. Each trough was tested for leaks by filling the center section with water. These wax partitions suited our purpose admirably since they were flexible and did not crack either when the soil was tamped in place or when the soil in the end sections was alternately watered and dried. The sections of the troughs were filled with Yolo clay, the same as that used in the previous experiment. In some of the troughs the center sections contained soil with a moisture content of about 11 per cent. which was less than the permanent wilting percentage. In others, the soil in the center section was wet throughout. The end sections were irrigated and sunflowers and beans were planted in them. The plants were watered at frequent intervals until the end of the experiment. The center sections were covered with a double seal of tin foil and plate glass set in putty to prevent evaporation. Since there was no significant change in moisture content of the dry soils in the center sections of the troughs during the course of the experiment, the indications are that roots were not present.



FIG. 1. Galvanized iron-lined troughs, divided into three sections which are separated by waxed partitions and contain wet and dry soil. The sunflowers and beans have been allowed to wilt.

Figure 1 shows four of the troughs 48 days after planting the seeds. The plants had been allowed to wilt in order to make sure that the roots had permeated the soil in the end sections. Figure 2 shows the same plants

within 2 hours after watering the soil. In one of the troughs, holes of about 6 mm. diameter were punched through the wax partitions for the purpose of insuring the passage of roots through the partition in case they were not able to penetrate the wax seal. The center section of the trough with the perforated partitions was filled with dry soil (11 per cent. moisture content) and no additions of water were made to it through-



FIG. 2. The same troughs and plants shown in figure 1 after the plants were revived by watering. The plants recovered within two hours.

out the experiment. To avoid the possibility of having water seep through the holes in the partitions into the dry center section, the plants were watered by means of porous clay cones or auto-irrigators of the type described by LIVINGSTON (4) installed as shown in figure 3. The surface of the soil in the end sections of the trough was irrigated with small amounts of water to start the plants growing but surface applications were discontinued as soon as the plants appeared above ground. Water thereafter was furnished by means of auto-irrigators.

The seeds were planted on March 14 and continued growing until May 1, 1930, a period of 48 days. At the end of this time the plants were cut off at the surface of the soil, the tin foil and glass covers were removed, and the troughs were cut with a hack-saw and the fronts turned down as shown in figure 3. The soil in the end and center sections was exposed and could be examined carefully for penetration of roots. Roots were found in those center sections that had been wetted, but not in those that had been kept

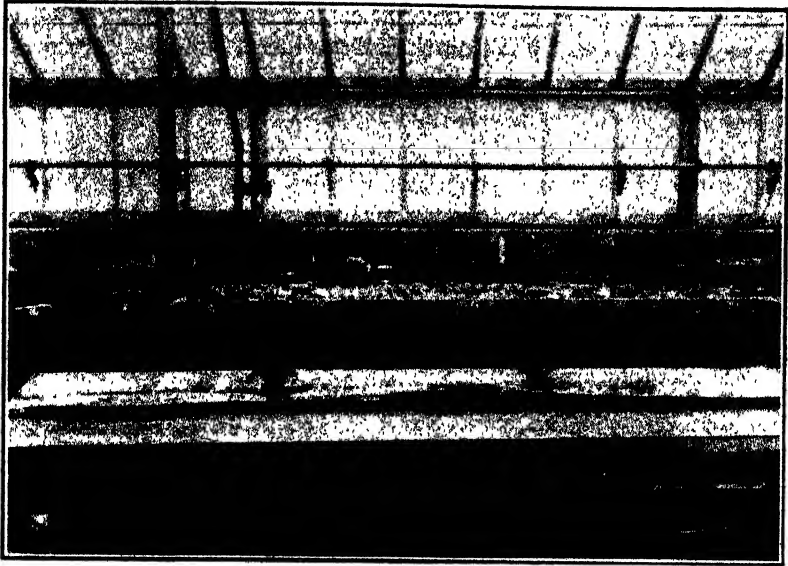


FIG. 3. Trough opened 48 days after planting the seeds. The center section was packed with dry soil and the end sections were watered with porous clay auto-irrigators. The method of supporting the waxed partitions is shown.



FIG. 4. Penetration of roots through the waxed partition when there was moist soil on both sides.

dry. Figure 4 shows roots which have penetrated the wax partition and have grown into the moist soil on the other side of the partition. The results of these tests indicate that roots will not grow into soil which contains less moisture than the permanent wilting percentage.



FIG. 5. The waxed wire basket with sunflower and bean plant which had been surrounded with dry soil for 47 days. Roots penetrated the wax seal but extended only a few millimeters into the dry soil.

A slightly different type of experiment was carried on at the same time as that in the troughs just described. A wire basket was made of hardware cloth and perforated plate bottom (fig. 5). The basket was coated with several layers of the wax, thus forming a flexible but water-tight con-



FIG. 6. The can containing the waxed wire basket with the plants was cut to expose soil. The roots came through the wax seal and grew into the soil on the outside of the basket which had been kept moistened for about 45 days.

tainer through which the roots could penetrate. The basket was then filled with the same kind of Yolo clay as that used in the troughs and was placed in a large sheet-metal can. Yolo clay with a moisture content of about 11 per cent. was packed around the basket. Water was added to the soil in the basket, but the soil outside was kept dry. Sunflower and bean seeds were planted in the basket on March 14, and after 47 days, the sunflower plants were as tall as those shown in figure 2. The plants were wilted which indicates that the roots had penetrated the entire soil mass within the basket. The basket was removed from the can to ascertain whether roots had been pushed into the dry soil which had surrounded the basket for a period of 47 days. Figure 5 shows the basket removed from the can. A few roots had penetrated the wax seal but had not pushed into the dry soil. The tips of these roots projected not over a few millimeters from the surface of the wax in about the same way that the roots had penetrated the wax seals in the troughs and in the 10-quart cans.

The wire basket was replaced in the can and again surrounded with dry soil. The soil inside the basket was watered, and the plants revived and continued to grow. Watering the soil inside the basket was discontinued after about two weeks and, instead, the soil on the outside of the basket was irrigated. The soil inside the basket dried out, but we were able to keep the plants from wilting by watering the soil outside.

On August 1, or about 45 days after the soil surrounding the wire basket was first wetted, the can was cut and the cut portion turned down to expose the soil as shown in figure 6. By August 1, the sunflower plants had matured and were cut off at the surface of the soil, but a bean plant remained. The roots had penetrated the wax seal and had grown out into the moist soil as evidenced by figure 6.

Distribution of roots as affected by auto-irrigators

The trough in which the porous clay auto-irrigators were installed also afforded us an opportunity to observe the distribution of roots as affected by these devices. Figure 7 shows that roots accumulate in the soil which is moistened in the immediate vicinity of the irrigators, but not in the other portions. There were many roots around the irrigators, a comparatively few between, but none were pushed through the perforated waxed partitions into the dry soil in the central section (fig. 3). As mentioned before, the surface of the soil in this trough was wet in order to start the plants growing, but thereafter water was supplied only by the auto-irrigators. Figure 7 also shows that only a relatively small portion of the soil was moistened by the irrigators. We believe the condition illustrated in figure 7 is typical of that which probably will be found in soils moistened by auto-irrigators.

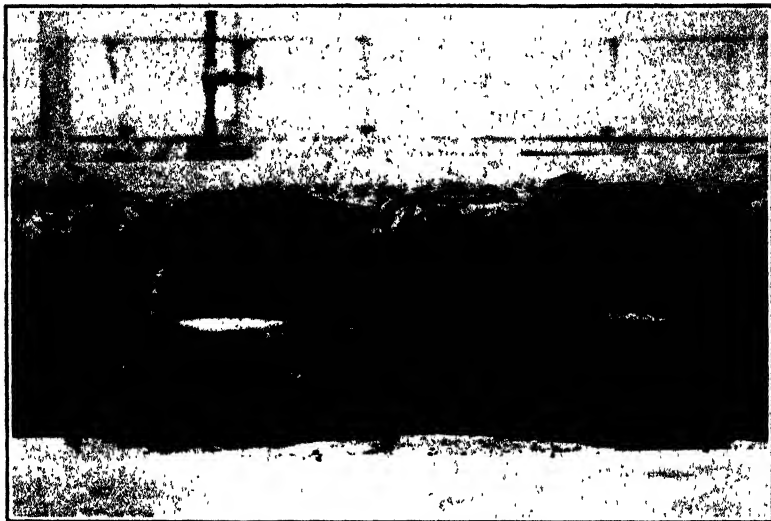


FIG. 7. Matted roots around the porous clay auto-irrigators.

These devices have been used by a number of investigators for the purpose of maintaining desired moisture contents of soils. The usual arrangement is to place a U tube containing mercury between the irrigator and the source of supply, as the moisture content of the soil is thought to be dependent upon the resistance against which the water must be drawn. We believe, however, that increasing the resistance simply results in decreasing the amount of soil wetted during the time usually allowed for growing plants, but does not result in wetting the soil in the container to a uniform moisture content, which is controlled by the resistance. SHAW (7), also, points out that the soil does not supply any of the "suction" force but functions the same as though it were lifting water from a free water surface. The development of roots of plants in containers at different soil-moisture contents could be studied accurately if it were possible to maintain a uniform soil-moisture content. However, we have been unable to maintain a uniform moisture content in soils on which plants are growing.

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COMPARISON OF METHODS OF DETERMINING MOISTURE IN CORN TISSUES¹

J. D. SAYRE AND V. H. MORRIS

(WITH ONE FIGURE)

Introduction

A series of studies on the physiology of the corn plant has involved a large number of moisture determinations. There has been wide variation in the moisture content of the various tissues and they undergo changes in moisture content along with seasonal development. Many of the measurements made in physiological studies involve calculations based either on dry weight or on the amount of sap in the tissue. It is important, therefore, to have simple rapid methods which will measure moisture content accurately.

Four different methods of determining moisture content have been used at different times and for different purposes. These methods involve sap expression, toluene distillation, air drying, and alcoholic extraction. Although the methods had been checked against each other several times it was desirable to carry out a more comprehensive comparison.

Material

A single variety of corn, Burr-Leaming, was used and the samples confined chiefly to leaf and lower-stem tissues, these differing widely in moisture content. The comparison was carried out with 16 samples taken on eight different dates from July 7 to Sept. 8, 1930.

The corn tissue was prepared for analysis by passing approximately one kg. through a food grinder. From this, comparable samples were weighed out and the moisture content determined in duplicate by the different methods.

Methods

Sap expression.—The total moisture content of fresh corn tissue may be determined conveniently in studies which involve the expression of cell sap. It is necessary, however, that the type of press cage used allow complete recovery either of the press cake or of the expressed sap. The type of hydraulic press and press cage used in this study has been previously

¹ Based on investigations cooperative between Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture and the Department of Agronomy, Ohio Agricultural Experiment Station.

described (4). In using this press 100 gm. of the samples were placed in the cage and the sap expressed at a pressure of 5,000 lb. per sq. in. The press cake was removed completely and weighed, the difference between this weight and 100 being the weight of the sap expressed. The quantity of moisture remaining in the press cake was determined by drying in an oven. The moisture content of the expressed sap was found by subtracting the total solids content as determined by a corrected refractometer reading from the total weight of sap expressed. The total moisture content of the original tissue then was the sum of the moisture remaining in the press cake and that in the expressed sap.

Toluene distillation.—A modification of the toluene distillation method, described by BIDWELL and STERLING (1) and applied to plant tissue by

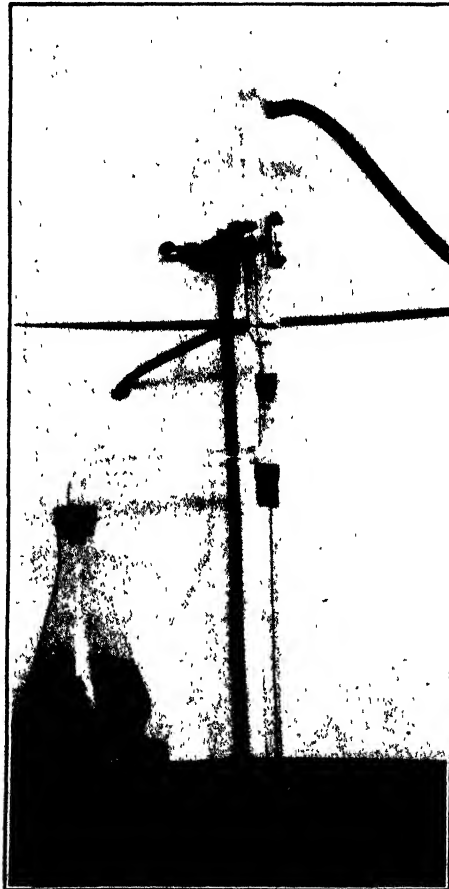


FIG. 1. Apparatus for determining moisture content of plant tissue by toluene distillation.

GILBERT (3), has been used extensively and successfully with corn tissue. The apparatus, modified for use with plant tissue containing large amounts of moisture, is shown in figure 1. In making the determination, 50 gm. of the sample were placed in a one-liter Erlenmeyer flask and covered with toluene. The flask was then connected to the apparatus for distillation and the contents of the flask brought to boiling over an electric ring heating unit. The mixture of toluene and water vapor passes through the tube into the condenser and, on condensing, drops into the receiver. This is a 50-ml. graduated cylinder calibrated in 2/10 ml. The water settles to the bottom and the quantity can be read directly.

Air drying.—The determination of moisture content by air drying was carried out by spreading a 50-gm. sample in a thin layer in a tray and placing it in a dry room maintained at about 48° C. After drying several days the residue was weighed and the moisture found by difference. It was necessary to use samples of at least 50 gm. due to the variability of the tissues, as well as to carry out a large number of determinations simultaneously. These conditions precluded the use of a small air oven or vacuum oven.

Alcoholic extraction.—In determining the moisture content of tissues preserved and extracted with 80 per cent. alcohol the usual procedure was followed, using 50-gm. samples. The total dry weight of the alcohol extract and that of the residue were determined and the moisture content found from the difference between the sum of these and the original green weight of the sample.

Results

The percentages of moisture as determined by the different methods are shown in table I, the duplicate determinations being entered in columns *a* and *b* under each method. The four methods were then compared with regard to: (1) agreement in determining the mean moisture content of all samples, (2) agreement in determining the moisture content over the entire range represented by the samples, and (3) the relative precision of the different methods in determining the moisture in duplicate samples. The comparisons are made in table II.

The mean of the 16 samples, as shown in table II, was 79.07 per cent. for sap expression, 79.18 per cent. for toluene distillation, 78.48 per cent. for air drying, and 78.95 per cent. for alcoholic extraction. The significance of the differences among these means was tested, using FISHER'S (2)

formula $t = \frac{x\sqrt{n}}{s}$ and the probability tables given by STUDENT (5). The odds were 332:1, 66:1, 332:1 against random differences as large as those between the means determined by air drying on the one hand and those

TABLE I
PERCENTAGES OF MOISTURE IN SAMPLES OF CORN TISSUE AS DETERMINED BY FOUR METHODS

DATE	TISSUE	SAP EXPRESSION		TOLUENE DISTILLATION		AIR DRYING		ALCOHOLIC EXTRACTION	
		(a) per cent.	(b) per cent.	(a) per cent.	(b) per cent.	(a) per cent.	(b) per cent.	(a) per cent.	(b) per cent.
July 7	Leaf	81.6	81.8	80.0	81.2	81.0	81.0	80.9	81.4
" 16	Leaf	77.6	77.5	79.6	79.6	77.0	77.2	78.3	78.0
" 22	Stem	89.8	90.4	89.8	90.0	90.0	90.0	90.4	90.4
	Leaf	76.9	76.2	76.8	77.4	75.6	75.6	76.3	76.6
" 29	Stem	89.5	90.2	89.4	89.0	90.2	90.2	90.0	90.6
	Leaf	78.1	77.4	77.2	77.0	76.0	77.0	77.4	77.4
Aug. 5	Rolled leaves	87.5	87.2	86.0	86.0	85.0	86.0	86.8	86.7
	Stem	84.9	84.6	86.8	86.4	85.2	84.8	86.3	86.6
	Leaf	70.7	70.1	70.4	70.6	70.2	70.2	71.0	71.4
" 18	Lower stem	84.0	83.9	82.2	82.2	84.2	84.4	84.2	84.1
	Leaf	70.2	70.5	71.8	70.8	68.8	68.8	68.7	68.6
" 25	Lower stem	77.2	77.0	77.8	77.6	76.8	76.8	77.5	77.4
	Leaf	71.4	71.8	70.8	70.8	70.4	69.8	69.5	69.6
Sept. 8	Lower stem	78.7	78.6	79.8	80.2	78.4	78.6	79.2	79.2
	Leaf	68.7	68.7	68.8	68.4	67.4	67.2	66.9	67.3
	Lower stem	78.3	79.2	79.6	79.6	79.2	78.4	78.9	79.0
	Mean	79.068		79.175		78.181		78.956	

TABLE II

COMPARISON OF FOUR METHODS OF DETERMINING MOISTURE CONTENT OF CORN TISSUE

METHOD	SAP EXPRESSION	TOLUENE DISTILLATION	AIR DRYING	ALCOHOL EXTRACTION
	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>
Mean moisture content	79.07	79.18	78.48	78.96
Significance of difference between means	Odds	Odds	Odds	Odds
Sap expression		2:1	332:1	2:1
Toluene distillation			66:1	4:1
Alcoholic extraction			332:1	
Product moment coeffi- cient of correlation	r	r	r	r
Sap expression		0.986 ± 0.005	0.995 ± 0.002	0.993 ± 0.003
Toluene distillation			0.988 ± 0.004	0.992 ± 0.003
Alcoholic extraction			0.997 ± 0.001	
	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>
Standard error	0.34	0.35	0.33	0.20

determined by sap expression, toluene distillation, and alcoholic extraction, respectively.

It is probable that the lower moisture contents indicated by air drying were due to the way in which the method was used. If the samples had been subjected to further drying at a higher temperature, or in a vacuum oven, better results might have been expected.

The differences among the means as determined by sap expression, by toluene distillation, and by alcoholic extraction were such as well might be due to chance, the odds not exceeding 4:1 in any comparison. It may be concluded, therefore, that these three methods are in excellent agreement.

The product moment coefficients of correlation for moisture content in the different samples as determined by the different methods ranged from 0.986 to 0.997. These are so close to perfect correlation as to show conclusively that the methods could be used interchangeably within the limits of variation as to kind and stage of tissues used which obtained in these experiments.

The standard error, $\sqrt{\frac{\sum d^2}{(m-1)(n-1)}}$, is used to measure the precision of the different methods. In this formula $\sum d^2$ indicates the sum of the squared deviations of the duplicates from their mean for each of the 16 samples; m is the number in a group, here the two of each pair; and n is the number of samples. As shown in table II, the standard error for the

alcoholic extraction method, 0.20 per cent. is less than that for the other three methods, *i.e.*, 0.34 per cent. for sap expression, 0.35 per cent. for toluene distillation, and 0.33 per cent. for air drying. Even these latter errors are less than one-half of one per cent. of the total moisture content; so all of the methods are satisfactory from this point of view.

Discussion

It is apparent from these results that choice among these methods of moisture determination for any particular physiological investigation will depend on their relative convenience in that investigation, the equipment available for the work, and the kind of tissue being used. The toluene distillation method can be used on any kind of tissue, while the sap expression method is limited to tissues from which the sap can be expressed. The alcoholic extraction and air drying methods can be used for any type of tissue. It is necessary to spread the material out in a thin layer for proper results by the air drying method.

If expressed sap is to be used for physical or chemical measurements, the moisture content of the tissue can be determined incidentally to that procedure, provided the right kind of press cage and hydraulic press are available. If plant tissue is to be analyzed by the alcoholic extraction method, the moisture content can be determined very accurately on the same samples. In other investigations, toluene distillation may be most convenient. Distillation may be immediate, in which case the data are available in less than three hours after sampling, or the flasks may be carefully stoppered after adding the toluene and distillation postponed indefinitely. Finally, in investigations in which the tissue is dried, as for mineral analysis, the moisture content can be determined nearly as accurately as by the other methods.

Summary

A comparison of four methods of determining the moisture content of corn tissue indicates:

1. The alcoholic extraction method was less variable than the sap expression, toluene distillation, or air drying methods.
2. The differences in moisture content due to differences in kind of tissue and to seasonal changes as measured by the four methods were almost perfectly correlated ($r = 0.986$ and over).
3. The air drying method indicated a significantly lower moisture content than any of the other methods. The differences were small, however, and may have resulted from the specific technique employed.
4. Sap expression, toluene distillation, and alcoholic extraction all indicated an almost identical moisture content within very narrow limits of random sampling.

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EFFECTS OF HIGH SOIL MOISTURE AND LACK OF SOIL AERATION UPON FRUITING BEHAVIOR OF YOUNG COTTON PLANTS¹

W. B. ALBERT AND G. M. ARMSTRONG

(WITH ONE FIGURE)

Introduction

An unusual shedding of very young cotton fruit buds was observed during the early part of the fruiting season in 1928 in the coastal section of South Carolina. This phenomenon was an important factor in limiting crop yields in many areas throughout that section. The young buds turned a distinct brown color and were aborted in most cases at the ages of one to five days. The greatest shedding of fruit buds usually occurs later in the season at ages of approximately six to eight days, the buds

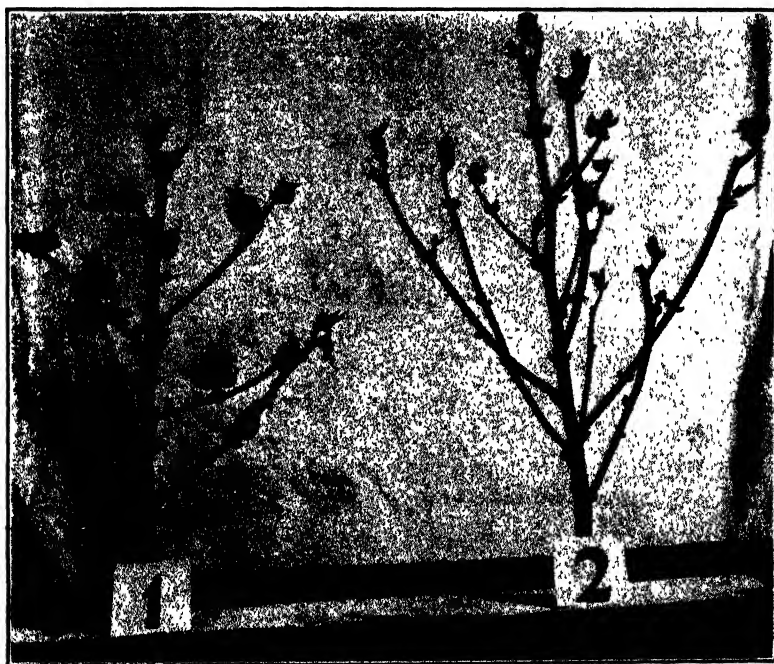


FIG. 1. Stalk no. 1 shows practically normal fruiting as compared with stalk no. 2 from which all early squares have aborted. Stalk no. 1 has one boll, 1 bloom, 28 squares. Stalk no. 2 has six squares.

¹ Technical contribution (New Series) no. 5 from the South Carolina Agricultural Experiment Station.

generally having a lighter green color than normal. Figure 1, taken from the Annual Report of the South Carolina Experiment Station for 1928 (1), shows an affected and a healthy plant. This injury is similar to that caused by the cotton flea hopper, *Psallus seriatus* Reuter, but repeated examinations by entomologists failed to reveal the presence of these insects.

Relatively low temperatures and frequent rains, which resulted in the soil being near the saturation point, occurred during the period of bud abortion. This suggested that poor soil aeration might influence materially the fruiting behavior of young cotton plants in the field. A survey of the effect of aeration on the root development of a variety of plants has been presented by CLEMENTS (5). CANNON (4) has pointed out the importance of temperatures in studying root behavior in various concentrations of oxygen. It was observed that normal root growth in several species of *Gossypium* occurred in soil atmospheres containing eight per cent. oxygen but that growth ceased when the concentrations of oxygen were below two per cent. Carbon dioxide injury to roots was related to the ages of the roots and also to the stage of development of the plant. WEAVER and CLEMENTS (8) present a general discussion of the importance of soil aeration to root growth. WEAVER and HIMMEL (9) report earlier production of flower stalks on *Typha* in a drained and aerated soil than in a water-logged soil.

The results reported in this paper were obtained from a study of some of the direct and indirect effects of high soil moisture upon the fruiting behavior of young cotton plants.

Experimental methods

Cotton was grown on two plats of Norfolk fine sandy loam soil, each with about 250 plants spaced 12 inches apart in four-foot rows. An untreated plat served as a check, the other plat receiving approximately two-thirds of an inch of tap water daily, except on rainy days when no tap water was applied. The tap water was applied with a hose in the afternoon. An attempt was made to keep the soil of the flooded plat at or near the saturation point. The fruiting record of each plant on both plats included the appearance of fruit buds and their subsequent shedding or production of bolls. The soil moisture content, hydrogen ion concentration, nitrate content, carbon dioxide and oxygen content of the soil air of both plats was obtained at various intervals. Tests for nitrites, ferrous and ferric iron of the soil solution were also made at various times. Colloidion bags as described by PIERRE and PARKER (6) were used to obtain diffusates for making nitrate determinations by the phenoldisulphonic acid method (2) and for hydrogen ion determinations by means of a Hellige

comparator. Soil air from the two plats was obtained with a device similar to that described by RUSSELL and APPLEYARD (7) and was always obtained at a depth of six inches.

The applications of water were begun at the appearance of the first fruit buds and were discontinued when appreciable numbers of blooms were present.

Results

The total fruit bud production and the percentage of bud and boll shedding at various periods are given in table I. It will be noted that the production of fruit buds was greater and the percentage of shedding less in the check plat than in the flooded plat, the total percentages of shedding being 8.0 and 15.1, respectively. The percentage of shedding was greatest

TABLE I
FRUIT BUD PRODUCTION AND BUD AND BOLL SHEDDING AT VARIOUS PERIODS

CONDITION OF PLANTS	FLOODED		CHECK	
	TOTAL NUMBERS	SHEDDING	TOTAL NUMBERS	SHEDDING
		<i>per cent.</i>		<i>per cent.</i>
Plants	238		202	
Fruit buds produced	5056		6337	
Fruit buds shed 1-10 days after appearance	248	4.9	207	3.3
Fruit buds shed 11-20 days after appearance	411	8.1	253	4.0
Fruit buds shed between 21 days and bloom	94	1.9	41	0.6
Bolls shed	10	0.2	3	0.05
Fruit buds and bolls shed, all ages	763	15.1	504	8.0

on both plats when fruit buds were between 10 and 20 days of age. It should be recalled that in 1928 the abundant shedding of fruit buds occurred when they were from one to five days of age. If the numbers of fruit buds shed at ages of one to 10 days in this experiment are calculated as percentages of the total which were shed, it is found that 32.5 per cent. were lost on the flooded plat in contrast to 41.1 per cent. on the control or check plat. After the fruit buds were 10 days of age, the percentage of shedding was greater on the flooded than on the check plat.

Table II gives the number of fruit buds that appeared during each period, the number that shed during these periods, and also the percentage

TABLE II

TOTAL NUMBER OF FRUIT BUDS PRODUCED, TOTAL NUMBER OF FRUIT BUDS SHED, AND PERCENTAGE OF SHEDDING FOR EACH PERIOD CALCULATED ON A BASIS OF 200 PLANTS

DATE (1929)	FLOODED			CHECK		
	NUMBER OF SQUARES PRODUCED	NUMBER OF SQUARES SHED	SQUARES SHED	NUMBER OF SQUARES PRODUCED	NUMBER OF SQUARES SHED	SQUARES SHED
			<i>per cent.</i>			<i>per cent.</i>
June 11 .	282	84	29.9	380	74	19.8
June 13	252	44	17.7	192	44	25.1
June 15	274	60	22.0	232	66	28.4
June 18 . . .	218	62	29.2	356	68	19.2
June 20	270	62	23.7	188	30	16.4
June 22	246	52	21.7	240	36	15.6
June 25 and 26	646	118	18.7	662	60	9.3
June 27 and 28 .	252	58	23.5	362	22	6.3
June 29 and July 1	294	32	11.4	442	21	4.9
July 2 and 3 .	236	22	9.9	698	21	3.1
July 5 and 6	276	18	6.4	758	24	3.4
July 8 and 9	436	19	4.4	776	11	1.5
July 10 and 11 .	496	8	1.7	748	3	1.6

of shedding. It will be noted that the percentage of shedding was appreciably greater on the flooded than on the check plat for each period with the exception of June 13 and June 15, which was shortly after the applications of water were begun.

Table III shows the percentages of moisture in the top soil of the contrasted plats, the percentages of oxygen and carbon dioxide in the soil air, and the percentages of shedding on each plat for the various periods. The percentages of oxygen in the soil air were consistently lower and the percentages of carbon dioxide higher in the flooded plat than in the check plat. The soil of the flooded plat usually contained more moisture than that of the check plat, the one notable exception occurring in the period June 27-28 when there was a heavy rainfall. Soil samples were taken just before the water was applied to the flooded plat, so that the percentages of soil moisture given in table III were the lowest to which the plants of that plat were subjected.

TABLE III
PERCENTAGES OF SOIL MOISTURE, CARBON DIOXIDE, AND OXYGEN CONTENT OF SOIL AIR OF FIELD PLATS COMPARED WITH THEIR SHEDDING PERCENTAGES OF VARIOUS PERIODS

DATE (1929)	FLOODED				CHECK			
	SOIL MOISTURE	O ₂ IN SOIL AIR	CO ₂ IN SOIL AIR	SHED- DING	SOIL MOISTURE	O ₂ IN SOIL AIR	CO ₂ IN SOIL AIR	SHED- DING
	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.
June 11				29.9				19.8
June 12	13.0	17.1	2.56		13.5	17.7	1.87	
June 13				17.7				25.1
June 14	14.2	14.5	1.87		12.0	19.8	1.53	
June 15				22.0				28.4
June 17	14.5	3.6	7.82		10.2	17.8	1.95	
June 18				29.2				19.2
June 19	14.9	10.7	4.93		12.0	17.1	3.23	
June 20				23.7				16.4
June 21	16.2	13.2	4.76		11.2	19.7	1.02	
June 22				21.7				15.6
June 23		13.0	4.93			20.2	0.85	
June 24	12.6				9.1			
June 25 and 26		11.9	2.89	18.7				9.3
June 27 and 28	16.3	10.5	2.89	23.5	17.7			6.3
June 29 and July 1	12.2	8.2	5.78	11.4	7.1	18.0	2.55	4.9
July 2 and 3	12.8	14.6	1.87	9.9	10.0	20.9	0.51	3.1
July 5 and 6	11.2	14.5	3.06	6.4	8.3	19.4	1.27	3.4
July 8 and 9	14.4	18.7	1.19	4.4	9.6	20.0	1.02	1.5
July 10 and 11	13.0			1.7	6.3			1.6

The hydrogen ion determinations and the nitrate analyses showed no significant differences. Nitrites were never present in more than traces. Tests for iron in the ferric and ferrous form in the collodion bag diffusate were always negative.

Experimental work on flooding carried out in greenhouse soil beds gave results similar to those presented, and the data are omitted.

Discussion

The shedding of fruit buds in this experiment did not occur at the same stages of bud development as that reported in 1928 (1), which suggests that the physiological responses of plants in this experiment were different from those of the field plants of 1928. Since warm, clear weather predominated throughout this experiment, in contrast to cool, cloudy weather in 1928, a different response might be expected. It should also be mentioned that the water-logging of the soil in this experiment did not occur until shortly after fruit buds appeared whereas this condition was present for some time before their appearance in 1928. The data show, however, that an increased percentage of fruit bud shedding and a retardation in plant growth were associated with poor soil aeration induced by the maintenance of a high moisture content. The percentage of oxygen in the unflooded soil air was only slightly less than that of normal air, in contrast with the soil air of the flooded plat which was appreciably lower than that of the atmosphere. According to CANNON (4), normal root growth will occur with an oxygen concentration of eight per cent. in the soil air. At only one time during this experiment was the concentration of oxygen in the flooded plat lower than eight per cent. The percentage of carbon dioxide in the soil air of the flooded plat varied considerably but was usually two to three times that of the check plat. It should be mentioned that samples of soil air were always obtained just before flooding, so that the percentage of carbon dioxide was probably at the lowest and the percentage of oxygen at the highest for the interval between times of sampling. The concentration of carbon dioxide in the soil air that interferes with the growth and functioning of the roots of cotton plants is not definitely known. The work cited by CLEMENTS (5) would seem to indicate that concentrations of three to five per cent. of carbon dioxide might influence root development and function, thus leading to the decrease in fruitfulness and the increase in fruit bud abscission which occurred in this experiment. BALLS (3) noted that raising the water table of irrigated Egyptian cotton fields was accompanied by an increased shedding of fruit. To determine more specifically the independent effects of soil aeration on cotton fruit bud development, experiments should be conducted in which temperature and light in addition to moisture and gas content of the culture medium are controlled.

Summary

1. A larger percentage of fruit buds was shed from cotton plants grown under conditions of high soil moisture content than from plants grown under more nearly optimum conditions of soil moisture.
2. The percentage of oxygen was lower and the percentage of carbon dioxide was higher in the soil of the flooded than of the unflooded plat.
3. A high percentage of carbon dioxide and a low percentage of oxygen in the soil air were associated with an increase in the percentage of shedding of fruit buds.

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BRIEF PAPER

PROOF OF THE ESSENTIAL NATURE OF COPPER FOR HIGHER GREEN PLANTS

(WITH TWO FIGURES)

In connection with experiments reported in this journal¹ by A. L. SOMMER and the senior author one experiment was conducted to determine whether or not copper is essential to the higher green plants. In that experiment flax was used as the experimental plant, and a series of cultures was run in culture solutions containing no copper with a parallel series the same in all respects except that 0.125 ppm. of Cu was added in the form of CuSO_4 . The plants in the two series grew equally well until the time of blossoming arrived, when it was noted that those in the series without Cu bloomed much less than the plants in the other series, but particularly that no plant deprived of Cu produced any seed capsule or seed. Since there were many plants in each series, and since every plant receiving Cu produced many seeds it appeared that the results were so striking as not to be accidental. Nevertheless, the senior author decided to run an experiment with another type of plant before reporting on the results. It did not prove feasible to start such an additional experiment until last fall. At that time the experiment described below was begun, and barley (*Hordeum vulgare*) instead of flax was tested. Since the experiment with barley was almost strictly parallel in its technique with that of the flax the details of the latter experiment will not be given, in the interest of economy of space; but the technique of the experiment with barley is given in detail, owing to the striking agreement of the results in the two cases, which prove, so far as we are aware, for the first time the definite need by plants for the element Cu, which must now be added to the steadily growing list in recent years of chemical elements essential to plants.

Experimental technique employed

The plants were grown in 2 liter Pyrex beakers in solutions of the following composition. Each beaker received:

KNO_3	1,600	milligrams
KH_2PO_4	300	"
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	1,000	"
NaCl	25	"
$\text{MnSO}_4 \cdot 7\text{H}_2\text{O}$	3	"
$\text{Al}_2(\text{SO}_4)_3$	1	"
ZnSO_4	1	"
H_3BO_3	1	"
CaSO_4	300	cc. of a saturated solution
Na_2SiO_3	2	cc. " " "
FeSO_4	as needed	

¹ PLANT PHYSIOL. 1: 1926.

The salts used were recrystallized several times and tested for the presence of Cu by the pyridine colorimetric method.² The limit of impurity with respect to Cu for each culture was determined to be less than 0.01 ppm. of Cu. The non-absorbent cotton plugs used to support each seedling showed no trace of Cu after remaining over night in dilute H_2SO_4 solution on the steam bath. The culture solutions were renewed once only during the experiment and *viz.* about 6.5 weeks after the commencement of the experiment.

Four barley seedlings were set out in each beaker and were approximately 6 cm. high at the start. Ten such beakers were used as controls to which no copper was added. Five beakers received each enough CuSO_4 to make a concentration of 0.0625 ppm. Cu (0.125 mg. Cu) and five more beakers received each enough CuSO_4 to make a concentration of 0.125 ppm. Cu (0.25 mg. Cu).

Observations on the cultures

The experiment was started on the 6th of November, 1930, and the following notes were made during the course thereof:

14th November. All plants in the three series look about the same. No evidence of toxicity of either concentration of Cu employed.

5th December. Plants receiving Cu seem to have longer and more vigorous roots than those receiving no Cu, but all plants and roots appear healthy.

23rd December. All culture solutions were renewed. New solutions were of the same composition as the old.

5th February. Plants in series receiving copper beginning to head out, but no heads in control cultures.

6th February. All plants in series receiving copper have heads, but only three plants in the control series show heads.

12th February. All plants have produced heads in all series.

20th February. No anthers visible in flowers of plants in control series, but plentiful in those of series receiving Cu.

20th March. Grain is well set in all plants receiving Cu, but apparently none in plants of the control series.

24th March. All plants were harvested.

During the period December to March the plants were attacked several times by aphids. Tobacco fumigation disposed quickly of these attacks. On the other hand, owing to many cloudy days during the same period, all plants suffered to some extent from attacks of mildew which, to avoid complications, was not treated. Artificial illumination, in addition to sunlight,

² Jour. Biol. Chem. 81: 1929.

was resorted to during January and part of February because of deficiency in sunshine and short periods of daylight.

Results of the experiment

In order to set forth briefly the results of the experiment we have arranged in table I the essential data. Comments on these follow.

The most striking feature of the data in table I is that dealing with head production in the barley plants. While apparently there is no significant difference as between the control plants and those receiving Cu in regard to the number of heads which emerged it is perfectly clear that the flowers of all plants in the control series were atrophied, whereas all plants receiving Cu produced good grain. In order to emphasize this point we accompany this paper with photographs, figures 1 and 2, which show some of the heads produced in each series of plants, the best and poorest heads having been chosen from each series so as to show the range of variability in addition to the main point of the demonstration. It will be noted that the heads in all series are small. This is attributable to the poor growing conditions of the winter season and the attacks of mildew on the plants, as well as to the impossibility of growing perfect plants to maturity in two liters

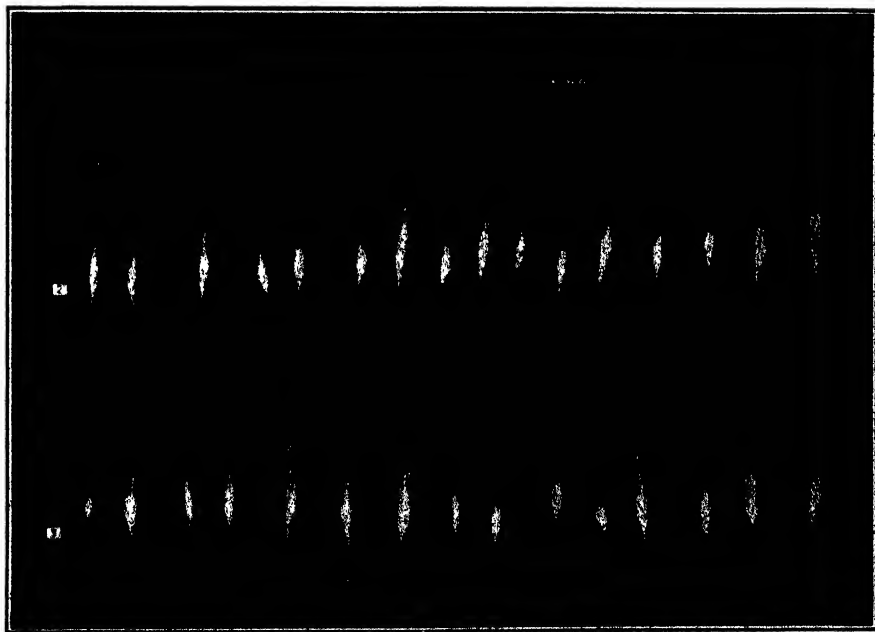


FIG. 1. Photograph showing the best and poorest heads produced in the absence of copper in the culture solution.

TABLE I
DATA ILLUSTRATING THE ESSENTIAL NATURE OF COPPER TO GREEN PLANTS

CULTURE NUMBER	TREATMENT	No. OF HEADS EMERGED	No. OF HEADS ATROPHIED	No. OF HEADS WITH GRAIN	AIR DRY WEIGHTS OF BARLEY PLANTS					TOP: ROOT RATIO
					Roots	TOPS LESS CULMS		WEIGHT OF CULMS	TOTAL WEIGHT	
						gm.	gm.			
1	No Cu	17	17	0	5.30	33.6	1.345	40.2	6.35	
2	" "	16	16	0	6.05	32.5	0.605	39.2	5.35	
3	" "	11	11	0	4.30	21.5	0	25.8	5.00	
4	" "	12	12	0	5.95	22.0	0.280	28.2	3.70	
5	" "	12	12	0	4.55	20.0	0.370	24.9	4.40	
6	" "	17	17	0	6.55	38.5	1.410	46.5	5.88	
7	" "	16	16	1 abort- tive seed	5.40	36.6	1.625	43.6	6.77	
8	" "	14	14	0	4.00	22.6	0	26.6	5.65	
9	" "	15	15	2 abort- tive seeds	6.90	35.7	1.710	44.3	5.20	
10	" "	14	14	0	4.45	26.1	0.450	31.0	5.86	
11	0.0625 ppm. Cu	15	4	11	5.345 \pm 0.413	23.0	2.705	35.03 \pm 3.9	5.42 \pm 0.333	
12	" "	15	5	12	5.05	22.2	4.195	30.8	4.55	
13	" "	15	1	14	7.35	28.0	6.530	33.7	3.03	
14	" "	19	0	19	7.40	29.8	5.060	41.9	3.78	
15	" "	19	0	14	6.75	24.4	10.065	41.6	4.41	
					7.95			42.4	3.07	
16	0.125 ppm. Cu	15	0	15	6.900 \pm 0.200	25.6	10.695	38.08 \pm 2.3	3.77 \pm 0.287	
17	" "	16	0	16	7.20	27.8	10.695	43.5	3.56	
18	" "	16	1 (slight)	16	7.70	23.3	8.515	47.5	3.02	
19	" "	15	0	15	7.75	26.5	9.215	39.5	3.03	
20	" "	15	1	14	6.95	22.0	6.250	43.5	3.42	
					7.76 \pm 0.141			35.2	3.16	
								41.85 \pm 1.7	3.24 \pm 0.050	



FIG. 2. Photograph showing the best and poorest heads produced with varying amounts of copper in the culture solution. (See table I.)

of solution changed only once in the growing season. But there is no room for doubt that barley plants (and as above indicated, also flax plants) will not produce seed without Cu in the root medium. The next point which should be emphasized in connection with the results in table I is the distinctly favorable effect exerted by Cu on root production. While the root and top production seems to be reversed in amount in the plants with and without Cu it should be noted that this is true only when the weight of the grain is not taken into account. Apparently the leaves and stalks of plants producing no grain contain more dry matter than those of plants producing good grain, but the weight of the grain is, on the other hand, infinitely greater in the plants receiving Cu than in those receiving no Cu. Even so, however, and in spite of the great variability in dry weight production between the individual cultures, the plants receiving Cu have produced more total dry matter than those not receiving it. It seems also to be beyond the pale of accident that as regards dry matter production the plants receiving the larger quantity of Cu produced more dry matter than those receiving the smaller quantity of Cu and also that this holds similarly for root production. Before proceeding to a summary of this experiment, attention should be drawn to the striking difference between the top-root ratios of the several cultures. The high top-root ratios in the control cul-

tures emphasize again the smaller root development in plants receiving no copper.

Discussion

The experiment described above proves conclusively that copper is essential to the welfare of the barley plant, and in view of the fact that similar results were obtained earlier with flax the presumption is strong that such a need for Cu is common to the higher green plants in general. While the results would seem to indicate the conclusion that Cu is necessary only for normal flowering and seed production, such a conclusion cannot be accepted without reservations. It must be remembered that the seeds in all series contained copper. The salts and the water used contained a trace of Cu, even though the salts were very pure and the water carefully redistilled before use. A trace of copper may have been present from solution of the glass. Most important of all, small quantities of Cu must have entered the solution from dust particles falling on the paraffined plaster of Paris covers used to hold the plants and cover the beakers. The appreciable quantities of Cu which must thus have been at the disposal of every culture whether Cu was added intentionally or not renders it probable that Cu is essential not only to seed production but to the growth of the plant in all stages. This view is somewhat strengthened by the behavior of the roots in the control cultures. We predict that it will be possible to show the essential nature of Cu to growth of the plant in any stage by exceptional purification of salts and water, by the total elimination of dust, and by the amputation of as much of the seed after germination as possible. It is highly probable that if Cu were needed by plants to the extent to which Zn and B are needed by them, it would be possible to show the effects of lack of Cu on plants to be parallel to those shown by SOMMER and LIPMAN in the paper cited above for the other elements.

It is singularly remarkable that with all the difficulties attaching to the technique of such experimentation it is still possible to show so strikingly the effects of the presence or lack of an element like Cu which plants require in such very small quantities, and when quantities of Cu above 0.25 ppm. in the medium have been shown in our earlier experiments to be distinctly toxic. The range in the amounts of Cu between those which are essential to plants and those which are toxic is very narrow indeed.

As regards the function of Cu in the plant cell, we are as much in the dark as we are about the specific functions of the other chemical elements which are essential to plants. There is ample evidence in the literature on the universal occurrence of Cu in plant tissue. We give conclusive evidence above that such presence of Cu in plants serves an essential purpose therein. But it still remains to be shown what essential purpose it serves.

In this respect we face one of the most difficult of many difficult problems in the mineral metabolism of plants. We are wholly ignorant of the specific functions of any of the essential chemical elements which do not themselves enter into the structure of molecules of organic matter.

Conclusions³

1. Barley plants are shown to be unable to produce seed without the presence of a small quantity of Cu in the root medium.
2. This does not prove necessarily that Cu is essential to seed production *per se*. Discussion is given to show that another conclusion is possible, namely, that Cu is essential to every phase of plant growth.
3. This confirms similar work carried out several years ago in this laboratory with flax plants with similar results.
4. One-sixteenth to one-eighth of a part per million of Cu in the root medium is sufficient to give the results noted above.
5. Other points pertinent to the experiment are discussed.—C. B. LIPMAN and G. MACKINNEY, *University of California*.

³ After the manuscript of this paper was in the hands of the editor we learned of the paper on the same subject by A. L. SOMMER which appeared in the April issue of PLANT PHYSIOLOGY. In view of the fact that we were not aware of DR. SOMMER's work, it is interesting to note the confirmation of these two investigations by each other. It is particularly interesting, in view of DR. SOMMER's results, to read again our discussion of our own results which was written without a knowledge of the results obtained by DR. SOMMER.

NOTES

Annual Election.—The Secretary of the American Society of Plant Physiologists has announced the election of the following officers for the year 1931–1932: President, Dr. W. E. TOTTINGHAM, University of Wisconsin; Vice-President, Dr. R. B. HARVEY, University of Minnesota. The Society should continue its splendid growth during the coming year with this vigorous leadership.

Pasadena Meeting.—The summer meeting of the Society, held at Pasadena in conjunction with the meeting of the American Association for the Advancement of Science, was well attended by members in the Pacific Coast and Rocky Mountain regions, and those reading papers were greeted by audiences numbering 60–80. Dr. D. R. HOAGLAND presided over the meeting. The arrangements were quite satisfactory except that a meeting of physiologists happened to be scheduled at the same time as a valuable symposium on photosynthesis and photochemical reactions held by the chemists. These occasional conflicts emphasize the desirability of having the programs of all sections and societies correlated, and coordinated, through some central agency. This sort of supervision might be exercised by the A. A. A. S., if the various societies did not feel that their rights were being invaded by such suggestive supervision. The central office is the only one which receives full information as to programs of all groups, and is, therefore, the logical source of help in avoidance of conflicts.

Pasadena provided perfect climatic conditions for the meetings, and the excursions to points of interest about Pasadena, Los Angeles, Hollywood, Santa Monica, the Mount Wilson Observatory, etc., were greatly enjoyed by everyone.

Committee on Incorporation.—A committee was appointed some time ago by Dr. H. R. KRAYBILL, President of the Society, to study the advantages and disadvantages involved in the incorporation of the American Society of Plant Physiologists. The committee consists of Dr. WALTER THOMAS, chairman, Dr. B. E. LIVINGSTON, and Dr. J. B. OVERTON. The committee is expected to report its findings at the next annual meeting, in New Orleans, and this report will serve as a basis for discussion and possible action at that time.

Lantern Slides of Plant Physiologists.—On various occasions attention has been called to the historical heritage of plant physiology, a priceless asset of this branch of botanical science. Several important steps have

been taken to build up a knowledge and appreciation of this history, and to create an interest in the great personalities associated with the rise of plant physiology to its present position.

It is appropriate to call attention here to the possibility of securing lantern slide portraits of some contemporary physiologists, as well as of the great leaders of the past. Institutions or individuals who may be interested in securing them at moderate cost, can select from a list of 80-100 names by writing to Dr. R. B. HARVEY, University Farm, St. Paul, Minnesota. The current cost is about 50 cents per slide.

Purdue University Section.—The Purdue University Section of the American Society of Plant Physiologists held their annual Spring dinner meeting on Tuesday evening, April 7, 1931. The local section had as the guest of the occasion, Dr. EDGAR NELSON TRANSEAU, Head of the Department of Botany, Ohio State University. Dr. TRANSEAU spoke on "The Prairie Peninsula," and illustrated his discussion with many lantern slides. The lecture was very interesting, and created much comment and discussion.

Agronomy Building at Wisconsin.—A four-story building is now under construction at the University of Wisconsin, to house the work of the Agronomy Department, the Department of Plant Pathology, and the State Seed Division. This building will be well equipped for graduate and research work, and the construction includes three new greenhouses. The Departments will occupy the building about September first.

Louis Hermann Pammel.—We regret very much to have to record the passing of another veteran botanist and member of the American Society of Plant Physiologists. Dr. PAMMEL died on March 23, 1931, as he was returning from California. A weak heart was the immediate cause of death.

Born at LaCrosse, Wisconsin, April 19, 1862, his boyhood days were spent on a farm in State Road Coulee near LaCrosse. In 1881 he entered the University of Wisconsin, and from this institution graduated in 1885 with a Bachelor's degree in agriculture. At that time Dr. WILLIAM TRELEASE was Professor of Botany at the University of Wisconsin. When Dr. TRELEASE went to the Missouri Botanic Garden Mr. PAMMEL accompanied him and served as his assistant from 1886 to 1889. In the latter year he received his Master's degree from Wisconsin, and came to Iowa State College as Professor of Botany. In 1892 he was made Botanist of the Agricultural Experiment Station. For more than 40 years he was connected with the Iowa institution, and for many years handled all phases

of botany, including bacteriology. At the time of his death, the staff of workers in his department numbered 37. In 1898 Professor PAMMEL received the Ph.D. degree from Washington University, St. Louis, and in 1925 the University of Wisconsin conferred upon him the degree of Doctor of Science.

Dr. PAMMEL was primarily interested in taxonomy, but his scientific endeavors included practically all fields of botany. Some of the earliest work on the physiology of seeds was done by Professor PAMMEL and his students. Dr. H. S. FAWCETT, of the University of California, was associated with Professor PAMMEL in this study. Professor PAMMEL was also interested in bacteriology. While at Wisconsin Doctor TRELEASE gave a series of lectures in bacteriology. He was the first to teach bacteriology in a land grant institution.

Dr. PAMMEL's most extensive publication was "A Manual of Poisonous Plants," published in 1911. He is also the author of Grasses of Iowa, Plant Ecology, Weeds of Farm and Garden, Weed Flora of Iowa, Honey Plants of Iowa. He had published numerous papers and bulletins on various botanical subjects.

Doctor PAMMEL was responsible for the establishment of various State Parks in Iowa. These parks have made it possible for thousands of his fellow citizens to enjoy more of the out-of-doors recreation, and only a few years before his death one of these parks was dedicated PAMMEL State Park in recognition of his services.

He was chairman of the Taxonomic Section of the Botanical Society of America in 1920 and in 1929. He also served as Vice-President of Section G, American Association for the Advancement of Science. Shortly after the American Society of Plant Physiologists was organized, he became a member of it. He took an active interest in Phi Kappa Phi, and was General Secretary of this body for a number of years; later he became its President General. He won for himself the admiration and respect of all botanists, and will be remembered as a tireless and inspiring worker for many good causes.

Plant Physiology.—A compendious textbook of Plant Physiology by Dr. EDWIN C. MILLER, of the Kansas State Agricultural College, has been published by McGraw-Hill Book Co. The work contains fourteen chapters, with the titles following: The plant cell; solutions and membranes in relation to the plant cell; the roots of plants; the intake of water by plants; the intake of solutes by the plant; the elements absorbed by the plant; the loss of water from plants; the formation of carbohydrates by the green plant; the nitrogen metabolism of the green plant; the process of digestion

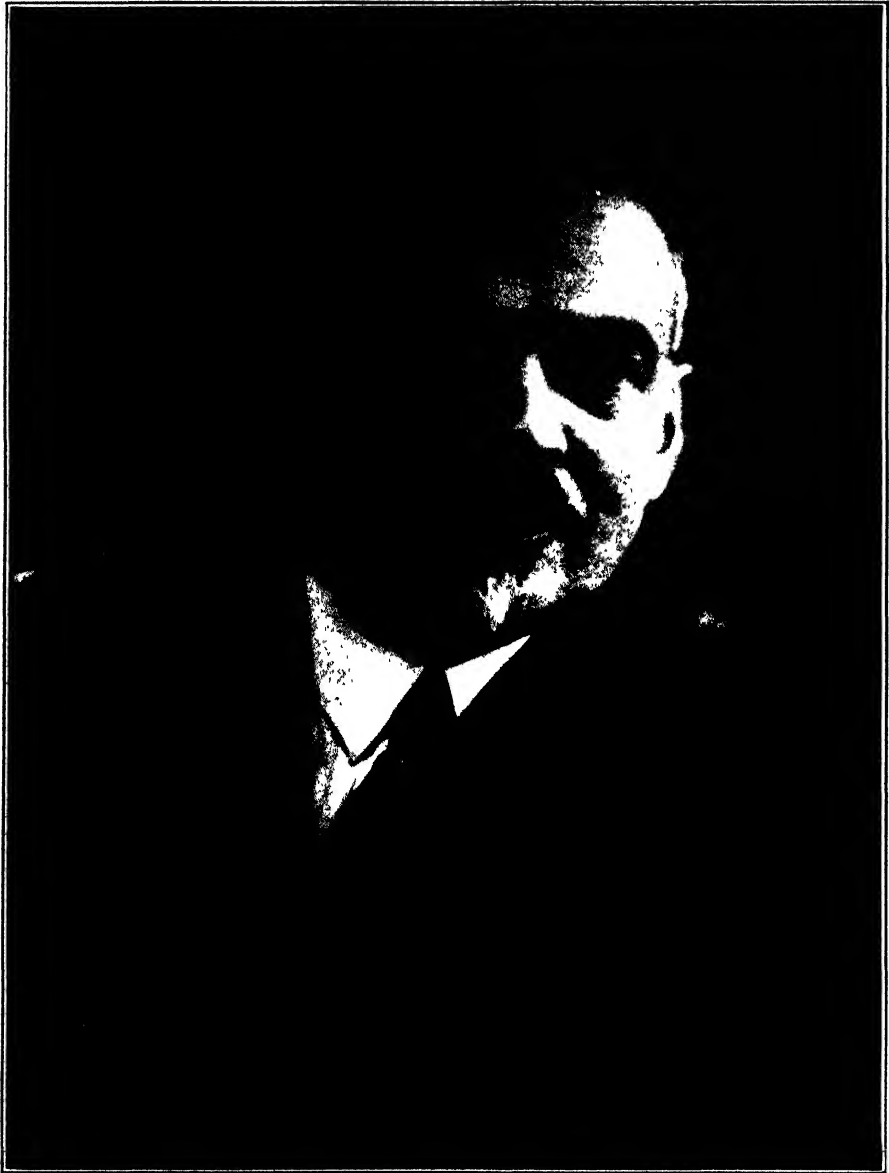
in the green plant; the translocation of materials in plants; the process of respiration in plants; and the process of growth in plants.

Stimulating questions are found at the end of each chapter, and hundreds of citations emphasize the fact that plant physiology is becoming too voluminous for any one to be able to read everything that is written about it, if he expects to do anything else than read. Author and subject indices are included.

The book is well written, and suitable for advanced students for reference. It is too compendious for an ordinary text, and the 900 pages has made the price (\$7.00) too high for general class use. Any one who has attempted to summarize this field will appreciate the enormous labor involved in Prof. MILLER's production; and those who read it thoughtfully will appreciate the fact that it has been done in a very praiseworthy manner.

Cell Stimulation.—Dr. METHODI POPOFF has written a book, "Die Zellstimulation," which has been published by Paul Parey, Berlin. In recent years POPOFF has worked with great energy in this field, and has stimulated a great deal of work among his students and colleagues. The introduction considers the theoretical conceptions underlying the work, while the body of the book is devoted to the experimental basis of POPOFF's theories. The three sections of work are entitled: I. Zellversuche; II. Tier- und Pflanzenversuche; and III. Samenstimulation. The problems are considered in relation to the application of the results in plant production and in medicine. There are 375 pages of text and 45 figures, in addition to the preface and contents. There is no index. The price quoted by the publisher is for paper binding only, 26 RM. Orders should be sent to Paul Parey, Berlin.

Crater Plants of Java.—Dr. HOWARD C. ABBOTT, Department of Biology, Evansville College, Evansville, Indiana, has translated Dr. FRIEDERICH C. VON FABER's "The Crater Plants of Java, in Physiological and Ecological Aspect." It is published in mimeographed form, in board binding, and can be obtained by writing to Economy Publishers, Mitchell, South Dakota. The price is \$1.60 per copy. Many sections are of physiological interest, such as the acid resistance of crater plants (sulphur dioxide), mycorrhizas, transpiration, power of absorption, nutritional activity of leaves, and chlorophyll content of crater plants. The original paper was from the Treub Laboratory in 1927.



NICOLAI ALEXANDROVITCH MAXIMOV

1880

DEPARTMENT OF PLANT PHYSIOLOGY
INSTITUTE OF APPLIED BOTANY AND
NEW CULTURES
LENINGRAD

PLANT PHYSIOLOGY

OCTOBER, 1931

EFFECTS OF CALCIUM DEFICIENCY ON NITRATE ABSORPTION AND ON METABOLISM IN TOMATO¹

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L. G. SCHERMERHORN

(WITH THREE FIGURES)

Introduction

This series of experiments was undertaken in an effort to determine the relation of calcium to the metabolism of carbohydrates and of nitrogenous compounds. Calcium is generally regarded as an essential element, and within recent years several workers (10, 17, 46, 55) have described a yellowing and a dwarfing of plants that had been grown in a deficiency of calcium, although they disagree as to the precise effect of such deficiency on the plant.

The tomato was used in the present experiments because it is well adapted to nutrient culture and because it has been used in so many experiments by different workers that a considerable knowledge of its nutrient requirements and metabolism is available.

Experimental methods

The experiments were carried on in the greenhouse at New Brunswick, New Jersey, during the early summer of 1930, with tomato plants of the variety Marglobe. On June 17 about 1,500 plants were selected for uniformity from 3,000 plants that had been grown for several weeks in sifted loam soil in four-inch pots. The plants were about 30 cm. in height, and were rather yellowish green in appearance; analysis showed them to be comparatively high in carbohydrates, but low in nitrogen (tables II, III, IV and V). The roots of each plant were washed free of soil, and the two lower leaves were removed; 300 of the plants were then used for initial

¹ The Kjeldahl and mineral determinations were made in the laboratory of C. S. CATHCART, for whose cooperation the authors wish to express their appreciation. They wish also to acknowledge their indebtedness to O. W. DAVIDSON for his help in obtaining photographs of plant material.

analysis, and the remaining 1,200 were transplanted for experimental treatments to washed quartz sand in new ten-inch clay pots, one or two plants to a pot. The pots were set in shallow enamel-ware pans and for the three weeks of the duration of the experiment all of the plants were subjected to nutrient treatments, each pot receiving daily two liters of nutrient solution. Twice a week each culture was thoroughly flushed with distilled water, and fresh nutrient solution was applied immediately.

The 1,200 plants were divided into four series according to the nutrient treatment which they received (see table I). One series was supplied with a complete nutrient solution that had been shown by previous experiments to be well adapted to the growth of tomato plants. The three other series received solutions lacking either calcium or nitrates or both. From time to time some of the plants were shifted from one nutrient treatment to another. The nutrient solutions employed all had a pH of about 4.7 when applied. After the solution had been in contact with the plant roots for 24 hours it was more alkaline, 6.2 to 6.4, but about the same in all series. The iron content of the initial plants or a trace of iron in the salts employed was sufficient for subsequent growth. Ferrous sulphate, however, was applied to some of the cultures in certain experimental trials described on page 617. Conditions of temperature and humidity during the progress of the experiments were suitable for the commercial production of tomatoes. The plants were grown under the seasonal light conditions of the greenhouse with the exception of some that were subjected to a period of continuous darkness at a practically constant temperature of 23° C.

Chemical methods

For macrochemical analyses, the plants were divided into seven fractions as shown in table VI. All terms are self-explanatory, except the term petioles, which in this paper includes also the rachis and the large veins of the leaf. Determinations of nitrogenous and carbohydrate fractions were made with fresh and dried tissues respectively, according to procedure previously described in detail (41). Aliquots of dried tissue were employed for mineral analyses (3).

Microchemical tests, which were made on fresh plant material, were in general those recommended by ECKERSON (12, 16), with modifications that will be described along with the presentation of results.

Results

On June 17, at the start of the various nutrient treatments, all of the plants were somewhat stiff and woody, with yellowish green leaves and a few blossoms. Macrochemical analysis showed that they contained no nitrates and no ammonia. They were very low in all forms of elaborated nitrogen but high in carbohydrates (tables II, III, IV, and V). Micro-

scopic examination of the stem showed a high percentage of very thick-walled mechanical and conductive tissue. Even though the plants were growing very slowly, cambium was active throughout the length of the stem. Starch was observed in large quantities in all parenchymatous tissue, especially in the pith, almost to the tip of the stem.

The initial plants contained the following percentage of calcium computed on a green weight basis; stems 0.13, blades 0.32, petioles 0.10, and roots 0.05. Calcium was present in three different forms: (1) calcium oxalate crystals, in the parenchyma of phloem, cortex, and pith; (2) "uncombined" calcium, *i.e.*, calcium that could be detected microchemically by the usual treatment with oxalic acid, and (3) "combined" calcium, *i.e.* calcium that could be detected microchemically only after treatment of fresh sections with a strong base such as NaOH. The terms "combined" and "uncombined" are purely relative, for, of course, all calcium in a cell is to some extent combined. "Combined" calcium was found in all living cells, not only along the walls but rather uniformly distributed through the protoplasts. "Uncombined" calcium was found throughout the protoplasts of all living cells. In collenchyma and parenchyma cells of the cortex and in parenchyma cells of the vascular cylinder, it occurred also along the walls, suggesting the presence of calcium pectate in the middle lamella. Many of the cells, however, especially of the pith, did not show a reaction which would indicate a middle lamella composed of calcium pectate in either plus- or minus-calcium plants.

PLANTS WHICH RECEIVED THE COMPLETE NUTRIENT SOLUTION

Throughout the course of the experiments (June 17–July 9) the plants that received the complete nutrient treatment exhibited a vigorous and



FIG. 1. Tomato plants—July 9, 1930. From left to right, plus-calcium plus-nitrate; minus-calcium plus-nitrate; and minus-calcium minus-nitrate.

apparently healthy growth of tops and roots, as shown in figures 1 and 2. Although these plants received a solution fairly high in concentration of nitrates (table 1), they were not soft nor extremely succulent. This was undoubtedly associated with the fact that the initial plants were hard,



FIG. 2. Roots of tomato plants—July 9, 1930. From left to right, plus-calcium plus-nitrate; minus-calcium plus-nitrate; and minus-calcium minus-nitrate.

high in carbohydrates and low in nitrogen (tables II, III, IV, and V), also that the experiments were run for a period of only three weeks. Dur-

TABLE I

COMPOSITION OF NUTRIENT SOLUTIONS. (PARTIAL VOLUME MOLECULAR CONCENTRATIONS OF SALTS USED)*

SOLUTION	$\text{Ca}(\text{NO}_3)_2$	KNO_3	KH_2PO_4	MgSO_4	CaCl_2
Plus-Ca plus- NO_3 ...	0.0180		0.0090	0.0090	
Minus-Ca plus- NO_3	0.0260	0.0090	0.0090	
Minus-Ca minus- NO_3	0.0090	0.0090	
Plus-Ca minus- NO_3 .		.	0.0090	0.0090	0.0180

* With the exception of the minus-Ca minus- NO_3 solution, each solution has a total osmotic concentration value of 2 atmospheres.

ing that time many fruits were set, and if the plants had been grown for a sufficient number of weeks undoubtedly they would have borne a heavy crop of fruit, as did the few plants which were allowed to remain.

During the progress of the experiments, certain factors remained practically constant in the various series of plants. The pH of the expressed sap of the various plant fractions, such as roots, lower stem, upper stem, stem tip, and blades showed little variation from one series to

another, although the usual (20) differences in pH of different plant fractions were observed. Microchemical tests failed to show any consistent outstanding variation in pH of respective tissues, although the usual differences (13, 41) were noted in pH of pith, xylem, phloem and cortex.

PLANTS DEFICIENT IN CALCIUM

In contrast to the plants that had received complete nutrient treatment, those that were grown from June 17 to July 9 in a solution lacking calcium (figs. 1 and 2) were stunted in vegetative growth and the stems were stiff and woody. Increase in volume occurred for only a brief period after the transfer from soil to sand on June 17. A few blossoms were formed but practically no fruit was set. The upper leaves were characteristically yellow, not yellowish green, whereas the lower leaves, which were thick and stiff, were still fairly dark green. Shortly after July 9 the stem tips of the calcium-deficient plants died.

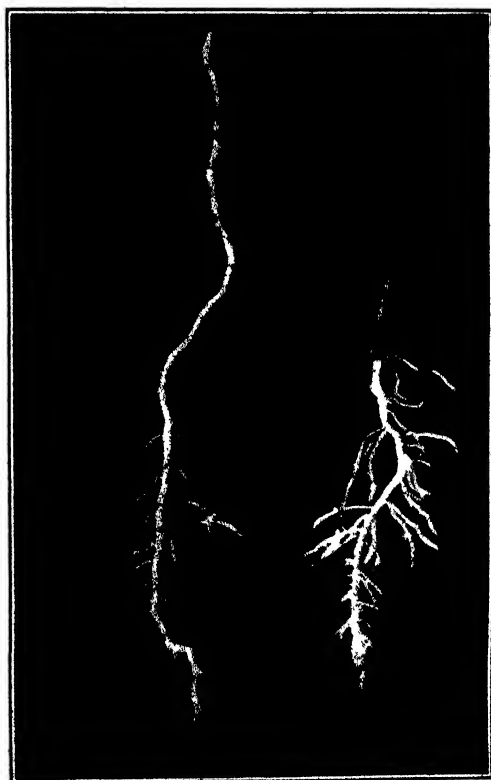


FIG. 3. Typical roots from tomato plants grown with the minus-calcium plus-nitrate nutrient treatment.

The leaves of some of the plants low in calcium exhibited a peppery brown spotting, apparently the result of iron toxicity rather than any direct effect of calcium deficiency or, on the other hand, of magnesium toxicity (page 618).

The root systems were short and much branched as shown in figures 2 and 3. Branch roots were short and stubby, and some showed characteristic browning of tips and of parenchyma further back, and sloughing off of some of the outermost cells. During the period of the experiments, however, the roots were alive and developed an abundance of apparently healthy root hairs.

The characteristics just noted were substantiated by macro-analysis and microchemical tests. Tables II and III show that the minus-calcium plants were even higher in carbohydrates than the plants that had received the complete nutrient treatment. Associated with the higher concentration of carbohydrates were thicker cell walls in the recently formed xylem.

TABLE II

CARBOHYDRATE FRACTIONS OF WHOLE STEMS EXPRESSED AS PERCENTAGE OF DRY MATTER, AND DRY MATTER EXPRESSED AS PERCENTAGE OF GREEN MATTER

DATE	JUNE 17	JULY 1		JULY 9		
TREATMENT	INITIAL PLANTS	PLUS-Ca	MINUS-Ca	PLUS-Ca	MINUS-Ca	MINUS-Ca DARK*
	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>
Dry matter	14.35	12.25	14.30	13.55	16.10	12.66
Reducing sugars	7.33	2.36	6.27	3.70	7.19	6.40
Sucrose	8.91	5.25	7.96	6.21	8.33	3.99
Total sugars	16.24	7.61	14.23	9.91	15.52	10.39
Starch and dextrin	23.74	8.92	19.17	10.04	25.73	16.54

* After July 1, some of the minus-Ca plants were subjected to continuous darkness.

TABLE III

CARBOHYDRATE FRACTIONS OF WHOLE STEMS EXPRESSED AS PERCENTAGE OF GREEN MATTER

DATE	JUNE 17	JULY 1		JULY 9		
TREATMENT	INITIAL PLANTS	PLUS-Ca	MINUS-Ca	PLUS-Ca	MINUS-Ca	MINUS-Ca DARK*
	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>
Reducing sugars	1.05	0.29	0.90	0.50	1.15	0.81
Sucrose	1.28	0.64	1.14	0.84	1.34	0.51
Total sugars	2.33	0.93	2.04	1.34	2.49	1.32
Starch and dextrin	3.41	1.09	2.74	1.36	4.14	2.09

* After July 1, some of the minus-Ca plants were subjected to continuous darkness.

Nitrates were comparatively low in the minus-calcium series, but on the basis of macrochemical determinations there was no significant difference between the calcium-deficient and the complete-nutrient plants in percentage or quality of organic nitrogen (tables IV and V). The quality of protein, however, was undoubtedly very different in the stems of the two lots of plants, as in the cells of the plants of the minus-calcium series,

TABLE IV

NITROGENOUS FRACTIONS OF WHOLE STEMS EXPRESSED AS PERCENTAGE OF DRY MATTER

DATE	JUNE 17	JULY 1		JULY 9		
TREATMENT	INITIAL PLANTS	PLUS-Ca	MINUS-Ca	PLUS-Ca	MINUS-Ca	MINUS-Ca DARK*
	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>
Total nitrate-free N .	0.553	1.530	1.375	1.609	1.649	1.570
Protein N	0.400	0.930	0.975	0.993	1.019	0.997
Nitrate-free soluble N	0.153	0.600	0.400	0.616	0.630	0.573
Basic N	0.020	0.045	0.044	0.119	0.128	0.130
Amino N	0.119	0.462	0.300	0.385	0.370	0.273
Amide N	trace	0.068	0.048	0.127	0.104	0.125
Ammonia N	none	trace	trace	0.002	0.002	0.003
Other N	0.014	0.025	0.008	-0.017	0.026	0.042
Nitrate N	none	0.040	0.025	0.211	0.086	0.270
Total N	0.553	1.570	1.400	1.820	1.735	1.840

* After July 1, some of the minus-Ca plants were subjected to continuous darkness.

TABLE V

NITROGENOUS FRACTIONS OF WHOLE STEMS EXPRESSED AS PERCENTAGE OF GREEN MATTER

DATE	JUNE 17	JULY 1		JULY 9		
TREATMENT	INITIAL PLANTS	PLUS-Ca	MINUS-Ca	PLUS-Ca	MINUS-Ca	MINUS-Ca DARK*
	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>
Total nitrate-free N .	0.079	0.187	0.197	0.218	0.265	0.199
Protein N	0.057	0.114	0.139	0.135	0.164	0.126
Nitrate-free soluble N	0.022	0.073	0.058	0.083	0.101	0.073
Basic N	0.003	0.006	0.006	0.016	0.021	0.016
Amino N	0.017	0.057	0.043	0.052	0.059	0.035
Amide N	trace	0.008	0.007	0.017	0.017	0.016
Ammonia N	none	trace	trace	trace	trace	trace +
Other N	0.002	0.002	0.002	-0.002	0.004	0.005
Nitrate N	none	0.005	0.003	0.028	0.014	0.034
Total N	0.079	0.192	0.200	0.246	0.279	0.233

* After July 1, some of the minus-Ca plants were subjected to continuous darkness.

particularly in the phloem region, but also in other parenchymatous tissue, there were granular inclusions that were not evident in the complete-nutrient plants. These inclusions gave positive protein reactions with all the usual reagents. Furthermore, such inclusions were conspicuous in the stem tips of calcium-deficient plants at the time of death of the meristem and shortly before.

Internally the stubby bulbous roots of the plants grown in the solution deficient in calcium showed differentiation of stele practically down to the embryonic tip, whereas in the faster growing roots of the complete-nutrient plants the rapid division and elongation of cells resulted in a long region of elongation between embryonic tip and zone of maturation. Within the stubby tips the planes of cell division were not irregular, although enlargement of cells was greater laterally than longitudinally. A factor which also contributed to the general appearance of stubbiness of the root system was the development of primordia of numerous branch roots, many of which never developed. Constrictions of the roots were not uncommon and were due to localized injury of outermost layers of cells and failure of these cells to enlarge properly, or to disintegration, or to both.

The externally conspicuous browning of roots of the calcium-deficient plants was almost entirely of parenchymatous and meristematic tissue. This was almost always observable first in the root tip, in the endodermis and in isolated cells of the cortex and stele. Later it occurred in practically all parenchymatous tissue, but did not appear to be present in conductive elements of xylem and phloem. Cellulose walls were chiefly concerned, but the protoplasts sometimes became decidedly granular, and this granular mass, as in the stems, was often a golden brown color and gave positive tests for protein with the usual reagents; the reaction with Millon's reagent was particularly strong.

The plants were tested for calcium from time to time during the course of the experiments. Microscopic examination showed the presence of some calcium oxalate in the base of the stem even at the end of the experiment, long after the plants had begun to show signs of calcium deficiency. The usual microchemical test with oxalic acid gave practically negative results, showing that there was little "uncombined" calcium present. However, an appreciably greater quantity was detected by treatment with oxalic acid after the sections had been treated with NaOH. These results suggest that the considerable quantity of calcium shown by macro-analysis to be present in the lower half of the tops (tables VI and VII), consisted largely of calcium oxalate deposits and of calcium so combined with protein or some other substance that it had to be released by hydrolysis before it could react with oxalic acid.

TABLE VI
MINERAL ANALYSES OF TOMATO PLANTS HARVESTED JULY 9
(RESULTS EXPRESSED AS PERCENTAGE OF DRY MATTER)

ELEMENT	CALCIUM		MAGNESIUM		PHOSPHORUS		POTASSIUM		SULPHUR	
	Plus-Ca	Minus-Ca	Plus-Ca	Minus-Ca	Plus-Ca	Minus-Ca	Plus-Ca	Minus-Ca	Plus-Ca	Minus-Ca
SERIES	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.
Upper blades	1.61	0.17	0.67	0.60	0.80	0.49	2.67	3.65	0.98	0.59
Lower blades	3.84	1.10	0.99	0.85	0.50	0.31	2.67	3.28	1.45	1.01
Upper petioles	1.08	0.11	0.92	0.23	0.73	0.78	6.27	9.64	0.38	0.51
Lower petioles	2.23	0.26	1.64	0.33	0.69	0.51	4.70	10.62	0.49	0.49
Upper stem	0.67	trace	0.69	0.24	0.70	0.68	5.25	6.02	0.33	0.31
Lower stem	0.99	0.53	0.50	0.30	0.41	0.43	2.27	4.39	0.28	0.25
Roots	1.26	0.25	0.46	0.60	2.34	0.72	2.23	3.18	0.75	0.55

TABLE VII
MINERAL ANALYSES OF TOMATO PLANTS HARVESTED JULY 9
(RESULTS EXPRESSED AS PERCENTAGE OF GREEN MATTER)

ELEMENT	CALCIUM		MAGNESIUM		PHOSPHORUS		POTASSIUM		SULPHUR	
	Plus-Ca	Minus-Ca	Plus-Ca	Minus-Ca	Plus-Ca	Minus-Ca	Plus-Ca	Minus-Ca	Plus-Ca	Minus-Ca
SERIES	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.
Upper blades	0.255	0.028	0.106	0.097	0.127	0.079	0.425	0.592	0.156	0.096
Lower blades	0.595	0.254	0.153	0.197	0.078	0.072	0.414	0.759	0.225	0.234
Upper petioles	0.935	0.010	0.080	0.021	0.063	0.074	0.543	0.909	0.033	0.048
Lower petioles	0.245	0.029	0.180	0.034	0.076	0.052	0.517	1.062	0.054	0.051
Upper stem	0.057	trace	0.059	0.030	0.060	0.085	0.452	0.759	0.028	0.042
Lower stem	0.150	0.087	0.076	0.050	0.062	0.071	0.345	0.724	0.043	0.041
Roots	0.149	0.035	0.054	0.084	0.276	0.101	0.263	0.447	0.089	0.077

PLANTS DEFICIENT IN CALCIUM AND NITRATES

The plants that throughout the experiments received a solution lacking in both calcium and nitrates, were in some respects like those that lacked calcium only. They too increased only slightly in size (even less than the minus-calcium series) and were woody in texture; microchemical tests showed that they too were high in starch and devoid of nitrates. Also, they contained practically no uncombined calcium but did contain a considerable quantity of combined calcium. However, they were not so yellow (fig. 1), and what yellowing occurred was chiefly of the older leaves, and not of the tips as observed above in the minus-calcium series. Moreover, the root systems were without the stubbiness of branch roots noted in the plants that lacked calcium only (fig. 3). This difference in appearance of roots and tops may have been due to the fact that with less vegetative extension there was a higher concentration of calcium per unit volume of plant tissue. It will be recalled that the vegetative growth of the plants of the calcium deficient series occurred for only a brief period after the start of the experiments. At that time all plants contained an abundance of calcium (page 607), were, in effect, plus-calcium plants, and were undoubtedly able to absorb and assimilate nitrates if present in the nutrient solution. On the other hand, in the series deprived of nitrates, no nitrates were available at the time of the initial growth of the other series of plants, when calcium was still available.

In an effort to study the absorption of nitrates, some of these plants deprived of both calcium and nitrates were shifted to the nutrient solution containing nitrates but lacking calcium. Hourly examination of these plants for nitrates, day and night for a period of five days after supplying nitrates, showed that during that period nitrates were not absorbed. Only after seven days was a minute trace of nitrates found in the roots, and later a little in the tops of the plants.

The fact that Dr. ECKERSON found practically no reducease in the roots of these plants nor in the roots of any of the calcium-deficient plants would seem to preclude the possibility of absorption and simultaneous complete assimilation of nitrates. Further, no nitrates and practically no ammonia were found in these plants at any time. In connection with reducease activity attention may be called to the relatively low phosphorus content of the roots of the calcium-deficient plants (tables VI and VII).

Although these plants of the minus-calcium minus-nitrate series did not absorb nitrates, they absorbed calcium instantly when shifted to the complete nutrient treatment. In forty-five minutes the entire root system contained uncombined calcium, and half an hour later there was a considerable quantity of calcium throughout the tops. It was, however, twelve

hours after the shift to plus-calcium treatment before these plants began to absorb nitrates from the solution, and about sixteen hours before faint traces of nitrites were observed.

DARKNESS TREATMENT

An attempt was made to study the hydrolysis and re-utilization of substances in darkness. On July 1, some of the plants that had been grown in the solution deficient in calcium only, were shifted from the greenhouse to a dark room that was maintained at about 23° C. The plants were kept in continuous darkness until July 9. During that period the stems elongated several inches, and the previously formed upper leaves turned from yellow to green, although newly formed leaves were yellow. As is indicated in tables IV and V there was an increase in concentration of nitrates. Further, microchemical tests showed that after the darkness treatment there was considerable uncombined calcium present, whereas other plants of the same series in the light contained practically no uncombined calcium.

It is apparent from tables II and III that carbohydrates decreased greatly during the period of darkness, yet there was much starch even in the tissues of the stem and leaves that were newly formed in darkness. The gross macrochemical analyses of stems (tables IV and V) show little change in percentage of protein or other elaborated nitrogen. There must have been, however, very definite changes in these substances, otherwise the rapid growth of meristem of the stem and the elongation of cells could not have occurred. Unfortunately macro-methods for proteins do not differentiate between those of the meristem and those of mature tissue. Microchemical tests showed, however, that during the period in darkness the granular masses of protein material (page 612) practically disappeared and much meristematic tissue of stems and roots developed. In these regions there were also observed appreciable quantities of asparagine.

On July 1, some of the plants that had been grown with no external supply of either calcium or nitrates were also placed in darkness. The same growth responses occurred. With the exception that nitrates were absent, similar chemical changes also took place, including the accumulation in the plants of considerable uncombined calcium that was not present in plants of the same series in the light.

After the darkness treatment, the plants (minus-calcium-minus-nitrate) were given a solution containing nitrates, but as before no calcium. Nitrates were observed in the small roots within fifteen minutes and throughout the plant within one hour. Such a rapid intake of nitrates was in striking contrast to the lack of absorption by plants of the same series in the light.

PLANTS DEFICIENT IN NITRATES

The fourth series of plants, those that were grown with no external supply of nitrates but with an abundance of calcium and other essential elements (plus-calcium-minus-nitrate solution, table I) were typical nitrogen-deficient plants (13, 27, 40). All the leaves, but especially the lower ones, were yellowish green with purple veins. The stems were hard and woody, very high in starch and free of nitrates.

These plants when shifted to minus-calcium-plus-nitrate treatment absorbed nitrates instantly. In less than an hour nitrates were present in all parts of the plant, and as usual (13, 40, 41) nitrites were observed in large quantities for three or four hours following the initial absorption of nitrates.

At the time of shift in nutrient treatments, the plants were observed for calcium distribution. Parenchymatous cells that were filled with masses of calcium oxalate crystals were conspicuous in phloem, cortex and pith. They were present in large numbers even in the extreme tip of the stem, and in parenchymatous cells of all parts of the root system. After transfer from the plus-calcium-minus-nitrate to the minus-calcium-plus-nitrate solution there was within a few days and for only a short time renewed vegetative growth. The concentration of uncombined calcium remained practically constant for about ten days, although there was a steady decrease in combined calcium during the same period. At the end of ten days, masses of calcium oxalate crystals were conspicuous in the base and middle of the stem, but none were present within five, and very few within ten, centimeters of the tip. The disappearance of calcium oxalate masses seemed to be first from the phloem and later from the cortex and pith. At the end of four weeks the concentration of combined calcium was very low, there was practically no uncombined calcium, and the stem tips were practically dead. Yet at that time calcium oxalate deposits were observed in the base of the stem and to a much lesser degree in the lower petioles. In many instances the masses looked as if they were undergoing decomposition.

Thus the re-utilization of calcium oxalate proceeded slowly and incompletely from the tip backward, and the plants died while crystals of calcium oxalate were still present in the base of the stem.

Discussion

If seedling tomato plants are transplanted to sand cultures deficient respectively in nitrogen, phosphorus, or potassium, the effects of nitrogen- or phosphorus-deficiency are evident in a comparatively short time (13, 40, 15), but conspicuous symptoms of lack of potassium (41) are not usually

apparent until much later. Although the symptoms of lack of these respective elements may not occur simultaneously, the effects upon the general appearance of tomato plants are similar. The lower leaves and lower stem are yellowish green tinged with the purplish blue of anthocyanin pigments, and the uppermost leaves and tip of the stem are fairly dark green and may remain so for a considerable period.

On the other hand, the appearance of the calcium-deficient plants of these experiments was very distinctive, unlike tomato plants deficient in nitrogen, phosphorus, or potassium, in that the upper part of the plant was yellow rather than green, and the lower half instead of being yellowish with practically dead leaves was fairly dark green.

Closely following the lack of chlorophyll in the tops, a condition which has been observed even in the lower plants (5, 17, 45, 46), there was a cessation of meristematic activity and death of tissue. This may, of course, occur when any essential element is deficient but it occurred in the tomato plants of these experiments within three weeks of minus-calcium treatment. Similar observations on tobacco plants were made by GARNER (17), who found that a lack of calcium affected most seriously the upper leaves and embryonic tissues of the growing point. He found that deficiency of magnesium, like deficiency of nitrogen, phosphorus or potassium, affected particularly the lower half of the plant.

The peppery brown spotting observed in the leaves of some of the calcium-deficient plants was probably iron toxicity, and was not due directly to insufficient calcium. Certainly it is not a distinguishing characteristic, although others (17, 43) have reported that these symptoms occurred when plants were grown in a deficiency of calcium. Analyses of leaf blades of the several series showed that there was often somewhat more filterable iron in the minus- than in the plus-calcium plants. Furthermore, the peppery spotting was easily obtained at will in plants of any of the series of these experiments but particularly in the calcium-deficient plants, simply by adding ferrous sulphate to the nutrient solution at the rate of ten or twenty milligrams of iron per liter. Respective tissues of calcium-deficient plants were not, however, more acid than those of the other series (page 608). If they had been, the higher percentage of soluble iron might be explained on the basis of SHIVE'S (53) results, for he has found that soluble iron is relatively high when cell sap is acid, relatively low when cell sap is more alkaline. On the other hand experimental work by SMYTH (54) has indicated that quality of acid may be an important factor in its influence upon the form of iron present, and work by GARNER (17) suggests that mineral deficiencies may directly or indirectly affect the quality of organic acids in a plant. In our experiments specific acids were not determined.

In view of popular theories (57) it also appeared possible that magnesium toxicity might be a factor in the growth responses obtained. However, when concentrations of magnesium sulphate more than three times as high as that of the regular nutrient solutions (table I) were applied for a period of two weeks to some of the plants not employed for analysis or other purposes, no spotting of the leaves nor any other visible effect upon the plants was observed in any of the series. The original total molecular concentration of the nutrient solutions was maintained by decreasing the concentration of potassium phosphate in proportion to the increase in magnesium sulphate. Likewise a few plants of the several series were grown for two weeks with no external supply of magnesium by substituting potassium sulphate for magnesium sulphate in equivalent concentration in the nutrient solution. The temporary removal of magnesium from the nutrient solution produced no visible effect on the growth responses of the plants. It is also apparent from tables VI and VII that there was no marked excess nor deficiency of magnesium in the minus- as compared with the plus-calcium plants. Further, PFEIFFER (42) shows that in oat plants the ratio of calcium to magnesium may vary within rather wide limits with no noticeable effect upon the plant.

It has been reported (10, 55) that lack of abundant calcium results in short, stubby, bulbous roots that are characteristically brown at the tips, with sloughing off of cells further back. The calcium-deficient tomato plants of these experiments were no exception. Internally there was differentiation of xylem and phloem practically down to the embryonic tip, which means, of course, that they were growing very slowly as compared with the plus-calcium roots, in which active division and elongation of cells resulted in a long region of elongation between embryonic tip and region of maturation. In the stubby tips of the calcium-deficient plants, however, distortions and irregular planes of division mentioned by SOROKIN and SOMMER (55) were not evident. The cells seemed entirely regular, although particularly in the cortex the cells enlarged somewhat more laterally than longitudinally.

The conspicuous browning of roots, a symptom very commonly associated with calcium deficiency, was due mainly to browning of meristem and parenchymatous tissue of the cortex. The stele seemed little affected and there were numerous root hairs that did not appear materially different from those of the plus-calcium plants except that in general they were somewhat shorter. The browning was mostly of the cell walls, which in healthy parenchyma are considered to be composed mainly of cellulose and pectic materials. The nature of change that resulted in browning of the cell walls was not determined. It is not uncommon, however, in tomato roots and may be produced by many types of injury.

No definite information was secured as to the presence or absence of a middle lamella of calcium pectate. After treatment with oxalic acid, rows of small calcium oxalate crystals were frequently observed along cell walls in the cortical tissue of roots of the plus-calcium plants, possibly indicating the presence of a middle lamella of calcium pectate. Although such a reaction was not invariably obtained in all cells even in the plants grown with an abundance of calcium, yet on the other hand, it was not observed at all in roots of the calcium-deficient plants that showed much browning and sloughing of outer cortical cells. It has, however, been reported by others (36, 45, 61) that in a deficiency of calcium a middle lamella of calcium pectate may not develop.

Whereas the browning of roots was mainly in cell walls, the protoplasts of the meristem and older tissue also became decidedly granular, and this mass was usually golden brown in color and gave strong protein reactions. Apparently in a deficiency of calcium, as in a lack of phosphorus (15) or potassium (41), there is definite interference with formation of proteins essential to the protoplasts of active cells.

Many different workers (4, 19, 22, 52), using various kinds of plants have found that carbohydrates frequently accumulate in the tissues of plants deficient in calcium. It has been shown, however, (page 617) that calcium limitation directly or indirectly interferes with chlorophyll formation. Therefore, under certain experimental conditions and in a deficiency of calcium the formation of chlorophyll might be so limited as to seriously decrease the rate of assimilation of carbon dioxide and thereby prevent accumulation of carbohydrates. This was possibly the case in results reported by BURRELL (6). Nevertheless, calcium deficiency seems quite commonly to result in carbohydrate accumulation, and the enormous concentration of sugars and starch in the low calcium plants of these experiments is clearly shown in tables II and III. Microchemical reactions also showed that starch was present in great abundance throughout all parenchymatous tissues, even in the tips of roots and stems.

In explanation of such observations it is usually said that calcium is essential for digestion of starch and translocation of sugars, and that in a lack of this element carbohydrates therefore accumulate. But it is evident that there is no sound basis for this notion, because in the calcium-deficient plants of these experiments sugar and even starch grains were found in the most distal portions of roots, stems and leaves. In what manner translocation of carbohydrates might be more complete is indeed a question. Also SCHIMPER (52) has shown that digestion of starch and translocation of sugars take place in leaves and stems of plants that contain a very low concentration of calcium. Further, the minus-calcium tomato plants of

these experiments after a few days in darkness decreased greatly in carbohydrates (tables II and III). Likewise, microchemical reactions showed the presence of an abundance of sugar and starch in tissues of stem and leaves that were yellow in color and newly formed in darkness. Thus there is additional evidence showing that there may be digestion of starch, translocation of sugars and re-condensation of sugars to starch in plants extremely low in calcium (tables II and III). It should be pointed out, however, that during the period in darkness there was an increase in uncombined calcium (page 615), although it does not appear probable that this was a direct factor in effecting digestion of starch and translocation of sugars, for the latter process began to occur considerably in advance of any apparent change in form of calcium.

One of the principal uses of carbohydrates is in protein synthesis. It has been shown repeatedly (13, 27, 39, 40) that carbohydrates may accumulate if a plant has no external supply of available nitrogenous nutrient. They may even accumulate with nitrates present in abundance in the nutrient medium or even in the tissue of the plant, if there is little reducase activity due to a deficiency of potassium (41) or phosphorus (15, 28). Both elements are necessary for synthesis of nitrates to protein.

When the plants were shifted from soil to sand culture at the beginning of these experiments all were in effect plus-calcium plants. At that time and for a short period thereafter, there was presumably some protein synthesis by the plants that were receiving minus-calcium nutrient treatment. However, carbohydrates undoubtedly accumulated in these plants because the amount of protein synthesis was small.

There are two reasons for the small amount of protein synthesis. First, the plants were very much limited in their ability to absorb nitrates (page 611), and second, the small quantity of nitrates absorbed (tables IV and V) remained for the most part unelaborated, as Dr. ECKERSON found that the calcium-deficient plants, especially the roots, were very low in reducase (nitrate reducing material). Apparently there was little synthesis of even the simpler organic compounds of nitrogen. The minus-calcium plants increased very little in volume. Accordingly, if there had been synthesis of simpler forms of organic nitrogen or protein it would be evident on a percentage-of-green-weight basis; but table V indicates that this did not occur. The effects of phosphorus (15, 28) or potassium (41) deficiency are somewhat different.

The concentration and quality of the various nitrogenous fractions of the whole stems were about the same in plus- as in minus-calcium plants (tables IV and V), yet microchemical reactions and anatomical examination showed decided differences. There was comparatively little meristematic

tissue in roots or stems of the calcium-deficient plants, but there were present in the region of phloem and inner cortex of roots and stems, cells which contained golden brown granular masses of material that was in large part proteinaceous. These protein-like inclusions may not be peculiar to calcium deficiency, as a somewhat similar condition may occur in a lack of phosphorus (15) or potassium (41), but apparently the very early death of embryonic tissue is a factor invariably accompanying a lack of calcium (10, 17, 52, 55). This element appears to be directly or indirectly necessary for development of proteins essential to the protoplasts of active cells. Without calcium, proteinaceous inclusions accumulate, not only in tomato but in other plants (58).

It is not apparent why a deficiency of calcium almost completely prevented absorption of nitrates (page 614), when the same plants that failed to absorb nitrates took in calcium instantly. The experimental results fail to indicate whether or not calcium deficiency limits the absorption of materials other than nitrates. Attention may, however, be called to tables VI and VII. The concentration of sulphur is much the same in both plus- and minus-calcium plants. Both lots of plants also contain about the same percentage of magnesium with the exception of the petioles of the calcium-deficient plants, which are comparatively low in this element. The concentration of potassium is comparatively high in the plants lacking calcium. This is a condition which has often been observed by others (31, 32, 47). It should be remembered, however, that the minus-calcium nutrient solution (table I) was comparatively high in potassium.

There seems to be nothing in the literature which has any very significant bearing on the inability of the minus-calcium plants to absorb nitrates,² although it is well known (18, 29, 30) that leguminous plants especially are high in nitrogen if the soil is abundantly supplied with calcium. The root systems of the low-calcium tomato plants were, of course, abnormal (pages 610 and 614) yet other essential elements were absorbed. The usual theories (57) on permeability are, in fact, opposed to the results obtained. Also, work by TRUE (61) and ECKERSON has shown that certain materials may be absorbed more readily by plants

² It seems impossible to draw conclusions from the work of JERMAKOW (23). He placed excised leaves in plus- and minus-calcium solutions and analyzed the leaf tissue for nitrates. At least as far as absorption is concerned, excised leaves can scarcely be compared with an intact root system. He found, however, a greater concentration of nitrates in the tissues of the minus-calcium leaves. He assumes, therefore, that there was more rapid assimilation (disappearance) of nitrates in the leaves which received an external supply of calcium. He did not, however, analyze his residual nutrient solutions, nor did he determine other nitrogenous fractions. He has therefore no measure of nitrates absorbed. Further, his leaves were presumably obtained from soil-grown plants, and it is doubtful if they were deficient in calcium.

deficient in calcium than by others in a full nutrient solution. These workers did not, however, determine the ability of a calcium-deficient plant to absorb nitrates.

Whether or not the low phosphorus content of the roots of the minus-calcium plants was a factor affecting nitrate absorption is not apparent. Only total phosphorus was determined.³ Although phosphatides are said to be important in determining the permeability of wall or protoplast (57) the question is at best unsettled (56), and little can be gained by discussion. It may, however, be pointed out that CHIBNALL (8) reports the presence of a calcium phosphatide in cabbage.

In connection with the low content of total phosphorus in the calcium-deficient roots, it may be emphasized that there rather than in the tops reducease was lowest. Also, ECKERSON's work (15) indicates that a lack of phosphorus limits reducease activity apparently more than a deficiency of other essential elements.

A series of excellent experiments by THERON (59) showed that the removal of nitrates was less from an alkaline than from an acid solution. Likewise SABININ and KOLOTORA (51) obtained somewhat similar results. However, in recent work (50, 60) with the tomato the pH of the nutrient medium did not materially influence absorption until the plant became apparently saturated with nitrates. In an alkaline medium, nitrate saturation within the plant occurred in a very short time; thereafter the plants did not absorb nitrates. The plants grown at a high pH had the ability to *absorb* nitrates but were unable to synthesize them to amino acids or protein.

The literature (9, 11, 37, 59, 62) is full of contradictory statements as to the effect of calcium deficiency on the pH of plants. Where whole tops are employed as a sample, the pH is usually lower in the calcium-deficient plants than in the more vigorously growing plants that have had an adequate supply of calcium; the difference can be accounted for by the fact that there is a larger amount of meristematic tissue, notably alkaline (20), in the latter. However, in homologous fractions of plus- and minus-calcium tomato plants the tissue and expressed sap were not materially different. The pH of nutrient solutions was also essentially the same in each of the respective series, practically optimum for maximum absorption and assimilation of nitrates (50, 60). Accordingly it does not seem probable that pH of plant or of nutrient solution was a limiting factor in the inability of the minus-calcium plants to absorb nitrates.

The failure of the calcium-deficient plants to absorb nitrates does not appear possible of explanation at this time. It is significant, however, that there was very rapid absorption of nitrates in low-calcium plants that had been subjected to a period of darkness (page 615). Within the minus-

³ Microchemical tests for calcium phosphate gave negative results.

calcium plants conspicuous changes were associated with darkness treatment; these included proteolysis involving the loss of many of the granular proteinaceous inclusions, decrease in combined calcium, and increase in uncombined calcium. RISSMAN (49) also records a decrease in concentration of insoluble calcium for wheat grown in darkness, and RAMANN'S (44) results indicate that there is more mobile calcium at night than during the day. The chemical significance of this change in form of calcium is not known. It is obvious that uncombined calcium alone (at least when newly absorbed) was not sufficient to result directly and immediately in nitrate absorption, for much uncombined calcium was present throughout the minus-calcium plants one hour after the shift to plus-calcium treatment. Nitrates, however, as determined by their complete absence and by lack of reduction products, were not absorbed until twelve hours later.

Apparently before nitrate absorption could take place other changes were necessary, changes probably involving the combination of calcium with protein or other materials. It is doubtful if proteolytic changes were a direct factor in either case, as only twelve hours of plus-calcium treatment were required before nitrates were absorbed. During such a short period of time there could scarcely have been extensive synthetic or proteolytic changes. But proteolysis or other catabolic changes in darkness released combined calcium. In another part of the experiment uncombined calcium was furnished by an external supply of soluble calcium salts. In both cases there apparently followed a re-combination with newly formed proteins or other materials. A supply of amino acids for formation of new protein was available in both instances; in the minus-calcium plants in darkness, through protein decomposition to amino acids, and in the plants newly supplied with calcium, through nitrate reduction to amino acids (page 615).

Non-absorption of nitrates was not a direct effect of a poorly proportioned nutrient solution, as the series of plants containing calcium but lacking nitrates (plus-calcium minus-nitrate, table I) absorbed nitrates instantly from the minus-calcium plus-nitrate solution.

Neither was darkness a direct factor in effecting nitrate absorption by the calcium-deficient plants. Moderate shading produced the same results but in lesser degree. Undoubtedly cloudy weather would tend to have the same effect, and probably the short days of winter in case of tomato (38, 41). All these conditions are apparently essentially similar in that they result in proteolysis, in a decrease in carbohydrates, and in the release of combined calcium for recombination with material of the protoplast. Likewise, if the initial plants of these experiments had been very low in carbohydrates and high in the simpler forms of organic nitrogen (40), a greater degree of re-utilization, or less combination of calcium might have taken

place and different results might have been obtained with respect to nitrate absorption. This point is to be investigated. However, a low concentration of organic nitrogen accompanied with a high percentage of carbohydrates in the plant does not in itself inhibit nitrate absorption. The plants of these experiments that were high in calcium and deficient in nitrates were low in all forms of nitrogen and extremely high in carbohydrates, yet they absorbed nitrate in abundance. But they also contained an abundance of uncombined calcium, which was available for combination with materials of newly formed cells. Also they were very high in reducase activity, as is usual for plants of such quality.

There appears to be but fragmentary evidence as to the forms of calcium found in plants, and much of this is misleading on account of the fact that air dried tissue has been employed for analysis (2, 25, 49). KOSTYTSCHEW and BERG (25) report that calcium does not occur in organic combination with protein. Their results, however, do not warrant this conclusion.⁴ On the other hand, there is apparently no proof that calcium combines with proteins, although in the body fluids of animals it is said (35) to form a non-ionized protein compound. It would, however, appear significant that increase in uncombined calcium closely paralleled changes in the minus-calcium tomato plants which must have involved proteolysis (page 615). There may, of course, have been significant changes in other materials such as possibly phosphatides of calcium. There is, however, increasing evidence tending to indicate that mineral elements may exist in combination with protein (24, 33, 35, 54, 63).

There are available many determinations (21, 48) of total water-soluble calcium. Such results, however, tell nothing as to the actual calcium compounds present. In many cases calcium oxalate undoubtedly accounts for much of the insoluble calcium fraction, and probably calcium phosphate under some conditions. Also much of the insoluble calcium is present as *combined calcium* (page 607). The proportion of soluble and insoluble calcium varies (21, 48), yet there seems always to be considerable insoluble calcium, whereas potassium is practically all soluble and readily translocated from mature to embryonic regions (41).

In calcium-deficient plants, however, it has been observed that most of the calcium present is in the older tissue of roots and tops (17, 21, 46, 61). Likewise, the low-calcium tomato plants (tables VI and VII) contained con-

⁴ KOSTYTSCHEW and BERG first dried plant tissue, apparently at room temperature, and extracted with cold water. Yet it has been shown by CHIBNALL (7) and others that such treatment results in extensive proteolytic changes; changes which in the tomato at least are associated with liberation of combined calcium. Following aqueous extraction these workers treated the tissue with 2N acetic acid and 2N hydrochloric acid. As might be anticipated, the ash of the residue was calcium-free, as either of the reagents employed react with the usually recognized calcium constituents of the plant.

siderable calcium in the lower half of the stem, yet there was only a trace in the upper stem. In this connection may be recalled the external appearance of the calcium-deficient plants, yellow in the upper half of the tops but green below. In contrast, in plants that are deficient in phosphorus (15), nitrogen (14, 27, 40), or potassium (41), the lower leaves are yellowish but the tips are comparatively green, and the greatest concentration of these elements is found in the tips or in other embryonic tissue (15, 27, 40, 41). Further, practically all the calcium present in fresh tissue of the minus-calcium tomato plants was found to be water-insoluble. Some of it was present as calcium oxalate deposits in mature tissues, but much of the insoluble calcium was in another form (combined calcium) that reacted with oxalic acid only after treating sections with alkali, or after the plants had been subjected for several days to continuous darkness or shading.

It has been shown by various workers (26, 34) that there may be to some extent re-utilization of calcium oxalate. The low-calcium tomato plants utilized calcium oxalate (page 616), but the crystals were dissolved by the plant so slowly that the embryonic tissue of stem and root tip died while there were yet heavy deposits of calcium oxalate in the base of the stem and in the lower petioles.

In addition to calcium oxalate crystals, there was combined calcium in older tissue of low-calcium plants when they died. Simultaneously, however, with disappearance of uncombined calcium there was death of meristematic tissue. When new cells are formed there must be not only calcium presumably for the middle lamella, but available calcium for combination with materials of the protoplast. It seems quite probable that some of this material may be protein, as without calcium inclusions accumulate in the protoplast in the form of granular masses that are, at least in large part, constituted of proteins (page 612).

Summary

1. The upper parts of the tops of calcium-deficient tomato plants were yellow and the lower leaves and stems were fairly green. (In a deficiency of nitrogen, phosphorus or potassium respectively, the lower portion of the plant is yellowish, but the upper leaves and stem remain green.)

2. The roots were characteristically short, bulbous and brown at the tips, with sloughing off of cells further back. The roots were short because of slow growth of the meristem, and bulbous because cortical cells enlarged somewhat more laterally than longitudinally. The failure of lateral root primordia to develop also contributed to the bulbous appearance.

3. Sloughing off of cells was probably due in part to the fact that a middle lamella of calcium pectate did not develop in roots of tomato plants extremely deficient in calcium.

4. Browning of the roots occurred in the cell wall and in the protoplast. The latter was composed in part of granular proteinaceous material. Such protoplasts were also present in the stem, especially near the meristem.

5. Calcium-deficient tomato plants of these experiments under the seasonal light conditions of the greenhouse were practically unable to absorb or assimilate nitrates although they absorbed calcium instantly.

6. The plants which lacked calcium accumulated carbohydrates in large quantities, apparently because absorption and assimilation of nitrates did not take place.

7. Translocation of sugars and digestion of starch took place freely in tomato plants extremely low in calcium.

8. Nearly 100 per cent. of the calcium of fresh tissue of the calcium-deficient plants was water-insoluble and most of it was located in older tissues of roots and tops. (In a deficiency of nitrogen, phosphorus, or potassium respectively, the highest concentration of the deficient elements is in young embryonic tissues.)

9. Some of this insoluble calcium was present as calcium oxalate, but much was in another form, *combined calcium*, that reacted with oxalic acid only after treatment with alkali.

10. In calcium-deficient tomato plants utilization of calcium oxalate and re-utilization of *combined calcium* took place, but so slowly that root and stem tips died while there were yet heavy deposits of calcium oxalate and a high concentration of *combined calcium* in older tissues.

11. When new cells are formed there must be calcium not only presumably for the middle lamella, but also available calcium for combination with materials of the protoplast. Otherwise granular proteinaceous inclusions accumulate.

12. Calcium-deficient tomato plants that were shaded or placed in continuous darkness for several days decreased in carbohydrates. Associated with the decrease in carbohydrates there was proteolysis and a noticeable increase in *uncombined calcium* and a diminished concentration of *combined calcium*.

13. Accompanying proteolysis and increase in *uncombined calcium* there was rapid formation of new stem tissue and absorption of nitrates, even though there was no external supply of calcium available.

14. Calcium-deficient plants in the light were given an external supply of soluble calcium salts. A few hours after absorption of calcium there was absorption and assimilation of nitrates, and subsequently growth was resumed.

15. Darkness treatment of calcium-deficient plants, and shift from minus- to plus-calcium nutrient, are apparently in certain respects similar in principle and effect. In both cases there is made available *uncombined calcium* for combination with newly formed proteins or other materials. A supply of amino acids for formation of new proteins of meristems is like-

wise available in both instances: in the calcium-deficient plants in darkness, through proteolysis, and in the plants newly supplied with calcium, through nitrate assimilation.

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ELECTRIC CORRELATION BETWEEN LIVING CELLS IN CORTEX AND WOOD IN THE DOUGLAS FIR

E. J. LUND

(WITH SEVEN FIGURES)

In presenting the results of the experiments which follow in this paper the writer will, for the sake of simplicity and proper evaluation of the observations, assume that the reader is familiar with the facts of electric polarity in the Douglas fir which have been presented in previous papers (LUND 1, 2, 3).

It will be recalled that when electrode contacts are made at the center of the wood and any point on the outer surface in the same cross-section of the tree, the center of the wood is always electropositive (in the external circuit) to the outer surface of the cortex. This is true for all regions of the tree stem below that of the first or second apical internode. Recent observations reported below indicate that the orientation of the radial polarity in the region of the most apical internode is reversed under conditions of absence of injury or stimulation.

Since all or at least the greater part of the radial P. D. is confined to the living parts of the wood-cortex system and since the cambium exhibits a bipolar structure which corresponds to the bipolar radial growth in wood and cortex, we might expect to find that the wood-cortex system including the cambium also exhibits a double electric polarity.

Up to the time of the present experiments and those to be presented in following papers on the effect of temperature, it had not been possible to demonstrate with certainty whether or not the cortex possessed an electric polarity of its own. The difficulty lay in the fact that any process of separation of the cortex from the wood axis involves more or less permanent injury, due to deformation of the cortex and therefore radical change in its electric polarity. This fact is also to be expected from the experiments reported in the preceding paper which pertain to the effect of mechanical stimulation on the electric polarity.

Various incidental observations seem to indicate that the radial E.M.F. *per se* in the wood is not affected to a noticeable degree by merely removing the cortex. This result might perhaps also be anticipated since no marked deformation of the wood occurs in such a process.

The preceding facts led the writer to devise a procedure by which the existence of the E.M.F. in the cortex could be made evident without injury or at least with a minimum of injury to the cortex.

The *principle of summation* of electric polarities of cells, which has been fully stated in previous papers, is obviously of fundamental impor-

tance. A proof of its validity and a demonstration of its rôle in electric correlation in the Douglas fir is one of the requirements, without which the writer's conception of the rôle of electric polarity of the cell in the process of correlation in tissues can not be considered established. The published evidence which appears to be conclusive is found in the experiments by MARSH (7, 8) on the onion root.

In the writer's first paper on the Douglas fir (LUND (1), pp. 10-11) and before we possessed any knowledge of the internal distribution of its correlation currents, it was assumed that the apparent algebraic summation of the external longitudinal E.M.F.'s along the stem was evidence for summation. This conclusion is of course correct provided it can be demonstrated that the origin of most or all of the observed external P.D. is not located at the surface of contact between the water leads of the electrodes and the external surface of the cortex. The experimental requirement was fully met in the experiments by MARSH on the onion root. In the following experiments it will now similarly be shown that the *removal of the cortex between the contacts without involving any disturbance of the contacts* themselves does produce characteristic and profound modifications of the E.M.F. similar to the results of the experiments by MARSH on the onion root. From these peculiar effects of the cortex on the E.M.F. we shall attempt to deduce in further detail the pattern of the correlation currents in the tree.

Experiments

The experiments reported here were carried out on the second and third internodes of the main axis of the tree. Each internode was isolated just before the beginning of the experiment. The lengths of the pieces varied from 50 to 70 cm. and the average diameters varied from 7 to 12 mm. The ratio of the length to the diameter was therefore actually about twice as great as that indicated in the diagrams of figure 1. Similarly in figure 6, the ratio of diameter to length of the internode is very much exaggerated for the purpose of illustration.

PROCEDURE I

Figure 1.1, curves, figure 2

In this experiment the third internode from a tree fifteen feet high was isolated and clamped rigidly at each end to a heavy iron stand. Dry cotton pads were wrapped around the jaws of the clamps to prevent mechanical injury to the tissues.

At points 10 cm. from each one of the cut ends of the internode, a ring of cortex 4 cm. wide, was removed. Holes two mm. in diameter were drilled to the center of the wood axis, at points midway between the cut

surfaces of the cortex. Into these holes were inserted glass funnels X and Z. The inner openings of the funnels were placed exactly in the center of the wood axis. These funnels were filled with tap water and served as electrode contacts at the center of the wood axis. Cotton strips saturated with tap water were wrapped around the wood axis and dipped into the cups A', A and B, B'. These cups were filled with tap water and two movable Pb-PbCl₂ electrodes were hung over the edges of any pair of cups between which a measurement of P.D. was to be made.

In all the work care was taken to prevent excess wetting of the exposed wood axis by the cotton contacts. These contacts and the cups X and Z were rinsed occasionally to maintain symmetry. Flowing contacts gave the same results as contacts which were rinsed occasionally.

In the experiment illustrated by figure 1.I, the P.D. was measured between each pair of the contacts, X and A', X and A, Z and B, Z and B', X and B, Z and A, A' and B', A and B.

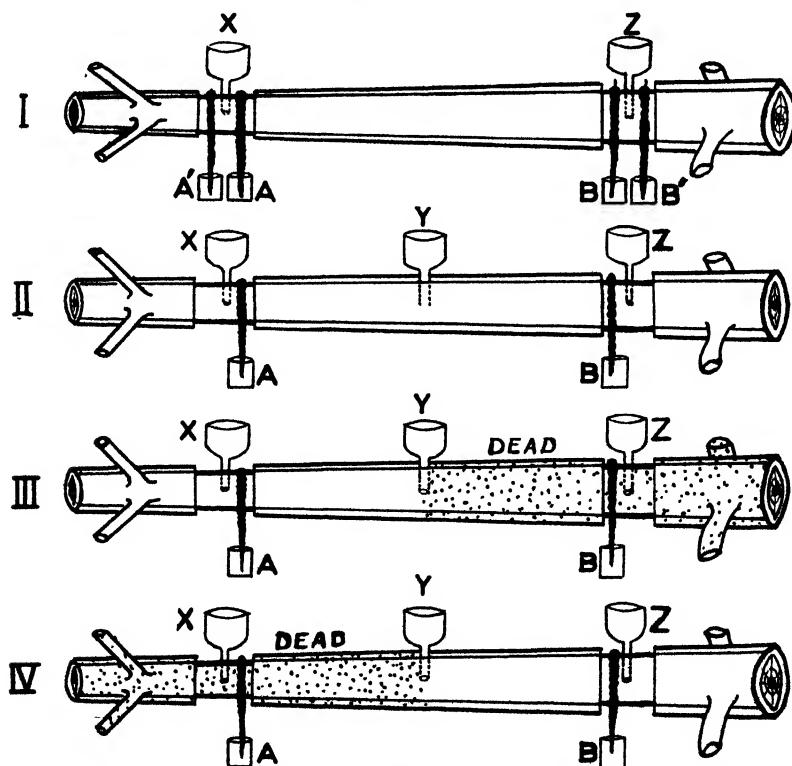


FIG. 1. Diagrams I, II, III and IV of an internode show the position of the glass funnel contacts X, Y and Z and the cotton contacts A and B in procedures of experiments I, II, III, IV respectively, which are referred to in the text. The stippled part of the stem was killed in boiling water before the tests were made.

The duration of one set of measurements by means of the potentiometer was about four to five minutes. The readings were always taken in the same sequence. Successive sets of readings were taken at variable intervals as indicated by the points on the curves in figure 2.

The following convention for designating the orientation of the electric polarity between the contacts will be followed throughout this and the following papers. The designations XB, AB, etc., on the curves mean respectively that contact X is electropositive in the external circuit to contact B; contact A is electropositive in the external circuit to contact B, etc., whenever the reading of the P.D. is above zero on the ordinate. Whenever the readings of P.D. fall below zero the contact designated by the first letter is electronegative to the contact designated by the second letter.

It will be observed in figure 1, and in the curves of figure 2 that:

- (1) XA', XA, and ZB, ZB' measure radial E.M.F.'s in the wood.
- (2) XB measures what will be called the apical-basal diagonal E.M.F.
- (3) ZA measures what will be called the basal-apical diagonal E.M.F.
- (4) AB and A'B' measure what we shall call the longitudinal E.M.F.

The first fact to notice is that the sequence in the magnitude of the radial E.M.F.'s of the wood at the four pairs of contacts is $ZB' > ZB > XA > XA'$. This relation is maintained during the whole period of the experiment and is merely a confirmation of previous measurements on the radial E.M.F. in which it was found that the radial E.M.F. of the wood diminishes as we proceed toward the apex of the tree.

In accordance with these facts we have arbitrarily designated the E.M.F. in the wood at the basal end of the internode in diagram figure 6 by 8 (\pm) signs and that in the apical end by 6 (\pm) signs. The significance of these arbitrary numbers will be referred to later.

The main object of the experiments is to show what effect the removal of the cortex between the contacts A and B has on the E.M.F. between each one of the different pairs of contacts. With this object in view three successive sets of measurements were made during the first thirty minutes of the experiment previous to removal of the cortex. During such a preliminary period there usually occurs a marked relative stabilization of the E.M.F.'s.

At the time designated by the vertical arrows the cortex between the contacts was gently but quickly stripped from the wood. During this process which lasted about two minutes the electrode contacts were not disturbed because the ends of the stem were fixed in rigid clamps. After the cortex was removed a reading was taken of each pair of contacts as designated in the curves, and thereafter the readings were taken at irregular intervals as indicated by the points on the curves.

INTERPRETATION OF THE CURVES

Figure 2

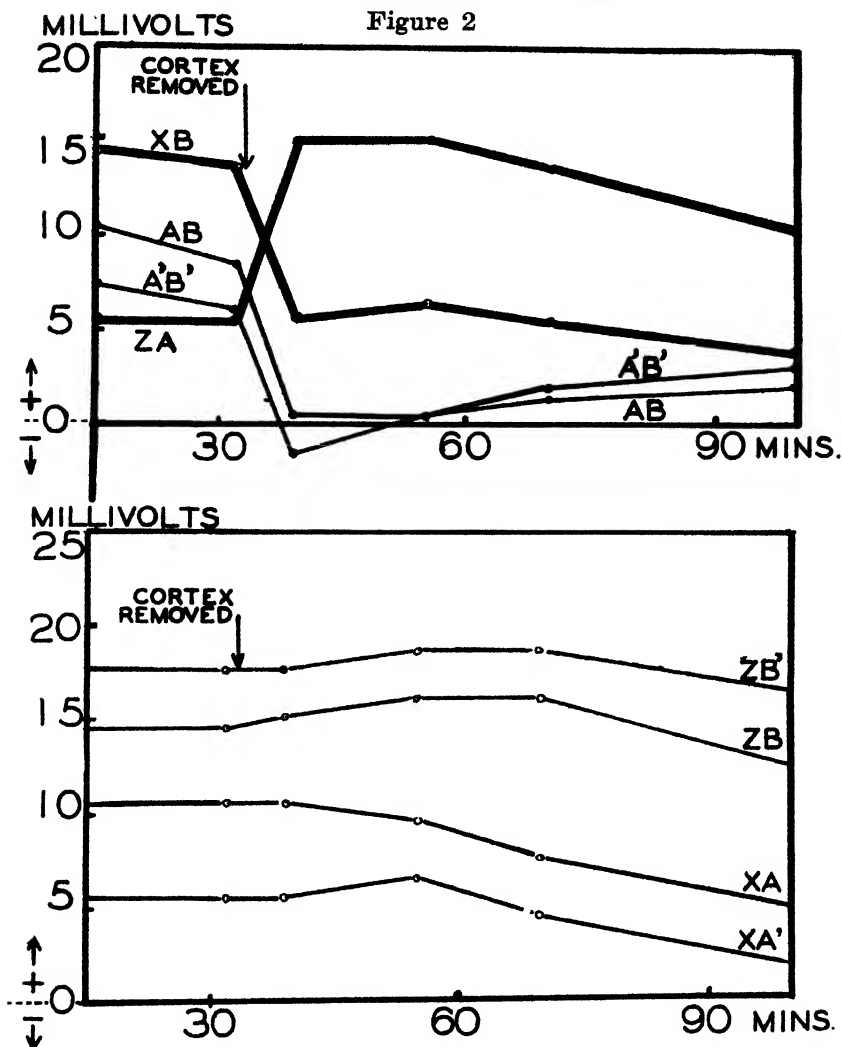


Fig. 2. Upper curves XB, ZA, AB and A'B' show the relative magnitudes of the E.M.F.'s between the contacts X and B, Z and A, A and B, A' and B' respectively, as shown in figure 1.I.

Lower curves ZB', ZB, XA, and XA' give the radial potentials of the wood between the correspondingly lettered contacts in figure 1.I. The upper and lower sets of curves are separated in order to avoid confusion. The magnitudes of the E.M.F.'s in the different curves should be compared.

The + or - sign of the potential on the ordinate axis always refers to the observed sign of the potential at the contact designated by the first letter. Thus in curve XB contact X is + to contact B in the external circuit. Similarly in curve A'B', contact A' is + to contact B' in the external circuit, except at one point on the curve where A' becomes - to B'. This method of designating the orientation of the E.M.F. is used in all the curves of the following figures. Position of arrows indicates the time when the living cortex was removed.

Curves ZB', ZB, XA and XA' show that under the conditions of this experiment, no marked effect is produced on the radial potentials of the wood when the cortex is removed. On the other hand curves AB and A'B' show that the removal of the cortex diminished the E.M.F. between contacts A and B, and A' and B', even though these contacts were not disturbed. If the origin of all the E.M.F. between the contacts A and B, and A' and B' was located at the contacts then certainly no change in E.M.F. would have been observed by removing the cortex. Since AB and A'B' are practically duplicates and therefore of the nature of controls for each other we can not regard the result as an accident. Furthermore all similar experiments on other internodes yielded the same kind of result. The drop in the curves AB and A'B' when the cortex was removed is in my experience only partially, if at all, ever recovered in such an experiment. The interpretation of this result must be that the origin of the E.M.F. in AB and A'B' is not merely due to a P.D. located at the electrode contacts, but that part of the total E.M.F. originates at loci along the stem between the electrodes. This constitutes important evidence for the validity of the principle of summation of E.M.F.'s in the stem. A permanent drop in the E.M.F. between A and B suggests the conclusion that the cortex is the seat of a permanent E.M.F. which, when the cortex is in position, in some peculiar manner augments (sums with) the longitudinal E.M.F. in the wood axis.

The most striking effect of removal of the cortex is on the E.M.F. between X and B, and Z and A. Note that XB is diminished while ZA is increased. These effects are quite permanent. Since the only difference between XB and ZA is that of an opposite orientation with respect to the polarity of the stem axis, removal of the cortex *must produce its opposite effects by virtue of the opposite orientation of the contacts with respect to the longitudinal axis of the stem*. This apparently means that the E.M.F. in the cortex, when *in situ*, *opposes* the larger E.M.F. of the wood between the contacts ZA and *augments* (adds to) the E.M.F. of the wood, between the contacts XB.

The experiment reveals the peculiar fact that the cortex is a tissue which determines in part the magnitudes of E.M.F. measured between contacts *on the wood*. The cortex appears therefore to be the seat of an inherent E.M.F. apart from the inherent E.M.F. in the wood. This fact is what we suspected to be true in previous papers but were not able to prove at that time. The above indirect procedure appears now to yield the answer.

For the sake of clearness and unity of thought we shall present at this time a semi-theoretical formulation of the facts. The following experiments will be used as tests for the validity of our interpretation.

The relative magnitudes of the electric polarities and direction of the resulting internal correlation currents are illustrated in the diagram of figure 6. The facts upon which this diagram is based are:

1. A radial E.M.F. with the orientation indicated by the + and - signs exists between center and surface of the wood axis.
2. The magnitude of this radial E.M.F. is less as the apex of the tree is approached. This is arbitrarily indicated by the numbers 8 and 6, which correspond respectively to the numbers of the + and - signs. For the moment we shall limit discussion to only two regions whose location is given by 6, 5 and 8, 2.
3. It will be shown later that there is a radial E.M.F. in the cortex, which is oriented oppositely to that in the wood, that is, the outer surface of the cortex is electropositive in the external circuit to the inner cambial surface. Furthermore this radial E.M.F. *increases* toward the apex of the tree. This fact is indicated by the numbers 5 and 2 in the cortex.

Now suppose we apply contacts at a and b, a current would then flow from a to b in the external circuit. This agrees with all actual experimental observations. Under natural conditions this external "return" circuit we will assume to exist in the conducting outer layers of the cortex. The main seat of E.M.F. is assumed to be in the inner young active phloem and cambium layers similar to the observed location of the greater part of the E.M.F. in the outer layers of the wood (LUND 3). The arrows in the cortex indicate the direction of the internal "return" circuits.

Again suppose we lead off from b and d, the direction of the flow of the current in the internal part of the circuit would be inward in the direction of the vertical arrow. This again is in agreement with experiment. Suppose we make contact at points c and d in the central axis, then the diagram shows that we would expect the current to flow in the *external* circuit from d to c. But now we must remember that since the electrical resistance of the old wood at the center of the tree is very high and the greater part of the E.M.F. designated by the numbers 8 and 6, lies near the surface in the young actively growing wood we would expect to observe a small current, and a small E.M.F. between the central points c and d as measured by the potentiometer. This is just what the experiments in previous papers have shown. The observed magnitude and orientation of the longitudinal E.M.F. in the center of the wood axis depends upon the position of the leads (= inner ends of the glass funnels) in the wood axis. The direction of the arrows at the center indicate the general direction of flow of the correlation current chiefly in that part of the wood having a relatively low electrical resistance.

Suppose we lead off from c and a, experiment shows again that c is electropositive in the external circuit to a, at least in the regions of the

second internode and in all internodes below it (*cf.* later data). Therefore the current in the cortex-wood system in such an observation would flow from a to c just as was observed between b and d. However, if we include the E.M.F.'s in both of the polar regions between a and c, and between b and d in a complete system and connect them by conductors, the direction of the current will be determined by the basal region b-d and not by a-c, because the E.M.F. of b-d is $8-2=6$ units while the E.M.F. of a-c is $6-5=1$ unit. These two are opposed to one another and the resultant E.M.F. will be the difference or 5 units of E.M.F. in our simplified case. The actual direction of flow of electric current in the region between a and c will therefore be that which is indicated by the arrow and not the opposite (*cf.* LUND (3), p. 277).

Regions a-c and b-d we speak of as being electrically correlated. In the above very simple case we may say that the E.M.F. of the region b-d determines more or less the direction of flow of electrical energy in the region a-c. In this manner it now becomes obvious that mutual interaction of E.M.F.'s may result in mutual modification of electrochemical processes and therefore mutual modification of other *linked* metabolic processes in different regions.

I wish to emphasize that the above formulation of the facts is to be thought of as a simplified description of the operation of the internal correlation mechanism. Without such simplification the presentation of the experimental facts becomes difficult and discouragingly indefinite.

Let us now return to the curves of figure 2. The fact that the longitudinal E.M.F.'s A'B' and AB are reduced by removal of the cortex, can now be understood from figure 6 in the following way. Since the inside surface of the cortex at b is more electropositive (=less electronegative) than the inside surface of the cortex at a, obviously this E.M.F. in the cortex when the latter is in its normal position, operates in *series* with the E.M.F. in the wood. That is, the E.M.F. between any two points in the longitudinal axis of the wood is increased by the presence of the cortex. Removal of the cortex would therefore be expected to result in a decrease in the longitudinal E.M.F. This agrees with the observed result.

The *resultant* E.M.F. of the cortex when in position, is oriented in the same way as the E.M.F. in the region d in the wood. Removal of the cortex should therefore cause a decrease in XB. The curve shows that it does.

On the other hand ZA is increased when the cortex is removed. This must mean that the E.M.F. in the wood in the region c is released from an opposing resultant E.M.F. residing in the cortex.

The opposite effects on XB and ZA caused by removal of the cortex, together with the fact that our diagram fulfills the requirements of observed

experimental facts, suggest very strongly that XB is, to a large extent, a measure of the E.M.F. in the region d-b and that ZA is largely a measure of the E.M.F. in the region a-c. The fact that the E.M.F. between c and d is usually small also suggests the same interpretation.

It appears to be evident from curves ZB', ZB and XA, XA' that the E.M.F. in the cortex does not affect these strictly radial potentials in the wood. In our interpretation of the experimental facts we have attempted to construct a diagram of the relations between the orientation and approximate relative magnitude of the internal correlation potentials in wood and cortex, which will fit the facts. The success to which we have attained will in part be indicated by the experiments which follow.

PROCEDURE II

Figure 1.II, curves, figure 3

The arrangement of the experiment indicated by diagram II in figure 1 is essentially the same as that in procedure I, except that another contact Y was added at the center of the wood and the contacts A' and B' were dispensed with.

The curves in figure 3 show that most of the potentials underwent a gradual decrease during the period of the experiment. When the drift (shift in flux equilibrium, cf. LUND (6)) had become uniform the cortex between A and B was removed. The time of removal is indicated by the vertical arrows.

The curves ZB and XA show again that the radial potentials in the exposed wood are affected little if any by removal of the cortex. Note also that $ZB > XA$. This is to be expected since the position of ZB is more basal than that of XA.

The E.M.F. of XB is decreased and that of ZA is increased by removal of the cortex. This result and its interpretation is again the same as that in procedure I above. It will be noted that this increase and decrease of E.M.F. resulting from removal of the cortex occurs in spite of the continual drift of the E.M.F.'s.

The longitudinal E.M.F. AB also drops to a very low value and remains low or absent during the remainder of the experiment. This result is the same as in procedure I.

The curves for the two measurements YB and YA are interesting for two reasons: First, because $YB > YA$. This indicates that YB measures largely the radial oblique E.M.F. located toward the basal region near the contact B, while YA measures the corresponding radial oblique E.M.F. in the apical region near the contact A. (cf. about p. 638). The second fact of interest is that *after* removal of the cortex, YB and YA *approach each other in magnitude*: Why? The answer is apparently given by our dia-

MILLIVOLTS

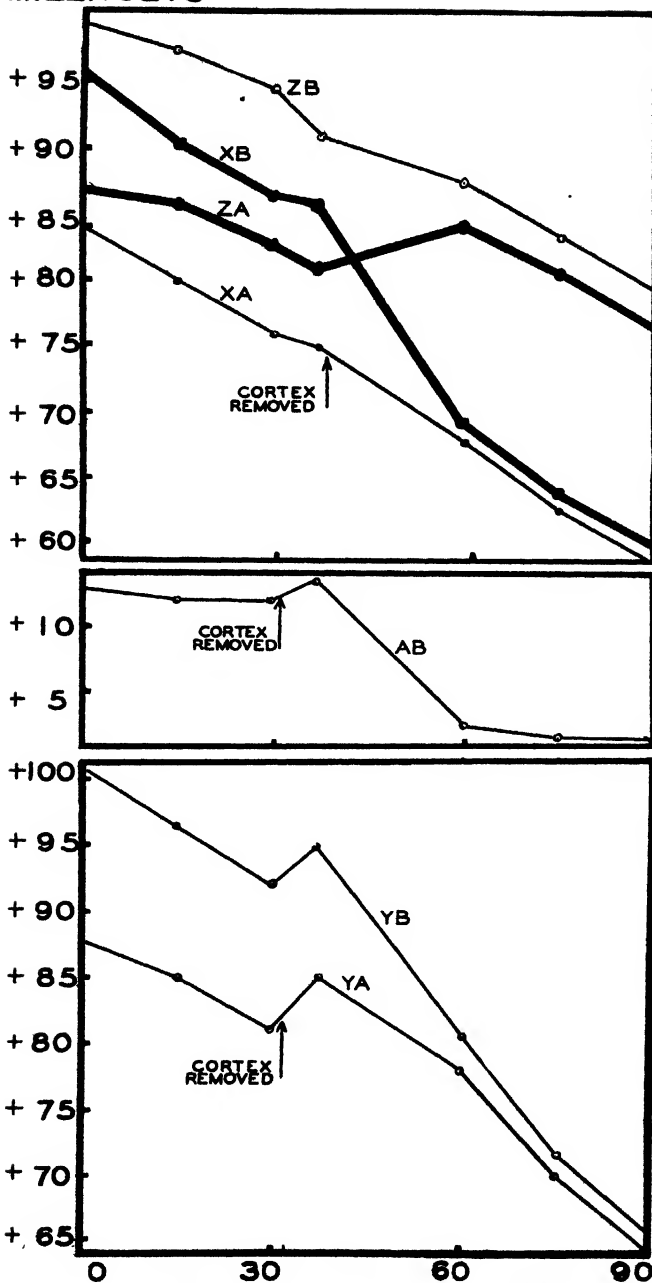


FIG. 3. Upper curves ZB, XB, ZA, and XA refer to E.M.F.'s between corresponding contacts in figure 1.II, and are strictly comparable to the curves in figure 2 designated by the same letters. The middle curve AB is strictly comparable to curve AB in figure 2. Curves YB and YA measure the E.M.F.'s between the corresponding contacts. Their relative magnitudes should be noted.

gram figure 6. Namely, the E.M.F. of the cortex when in contact with the wood, is applied so as to augment the difference in E.M.F. between the regions d and c, namely $8-6=2$. In order to augment this apparent difference (= difference between YB and YA) the E.M.F. at d (= 8) is increased, while that at c (= 6) is decreased. Or stated in other words the *resultant* E.M.F. of the cortex sums with the local and relatively basal radial E.M.F.'s in the wood since they are oriented in the same direction and are therefore in series, while relatively apical and local radial E.M.F.'s in the wood are decreased because the resultant E.M.F. of the cortex opposes the inherent apical radial E.M.F. in, for example, the region c. A second experiment of this kind gave the same results. Our diagram in figure 6 appears to be in striking agreement with the facts.

PROCEDURE III

Figure 1.III, curves, figure 4

EFFECT OF KILLING THE BASAL HALF OF THE INTERNODE BY HEAT.—The second internode from a tree twenty feet high was used. The total length of the internode was 50 cm. The apical and basal diameters of the wood at A and B were 8.5 and 12 mm. respectively. The lengths of the cortex between Y and A, and between Y and B, were 14.5 and 14.0 cm. respectively. The length of each ring of cortex which was removed at A and B was 4 cm. The set up in procedure III was identical with that in procedure II except that the basal half of the internode (stippled) was immersed in boiling tap water for two minutes. The heated cortex assumed a brown color.

During exposure of the basal half of the internode to the boiling water, the apical half was protected from injury by wrapping it in cloths soaked with cool water. The heated half of the internode was now cooled in running tap water, wiped dry and fixed in position with the contacts. The preparation was left for over an hour, during which several readings of P.D. were taken. Toward the end of this period the P.D.'s became relatively constant.

When this had occurred, the dead cortex on the basal half of the internode was quickly removed. The time of removal is indicated by arrows in figure 4. A set of measurements was taken and immediately after these readings, the living cortex on the apical half of the internode was also removed. Readings of E.M.F. were taken thereafter at intervals as indicated on the curves.

The effects produced by removal of the cortex in this type of experiment appear somewhat bewildering at first sight. A study of each curve by itself is however quite illuminating and reveals a number of interesting facts. The first fact which is apparently indicated in the curves is that

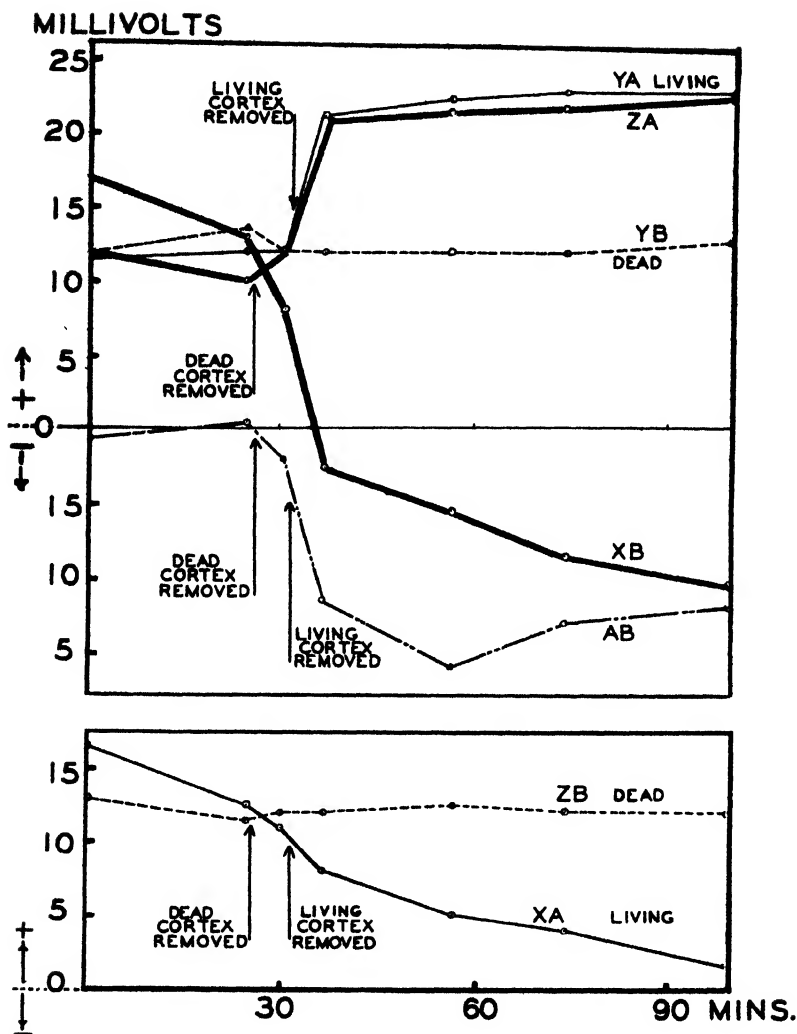


FIG. 4. Upper curves show the changes in E.M.F. produced by removal of basal (dead) and apical (living) cortex between the different pairs of contacts in figure 1.III. Lower curves are drawn on separate ordinates to avoid confusion. For interpretation see text.

removal of the living apical half of the cortex produces quicker and greater change in certain E.M.F.'s than the removal of the dead cortex.

Curve ZB which represents the radial E.M.F. in the dead wood is constant and has the same orientation as the E.M.F. in the living tissue. Similarly YB has the same orientation but not a greater magnitude relative to YA in the living apical half, as would have been found if the region YB

were living.¹ Curve XA of the radial E.M.F. of the living wood shows a drift in the E.M.F. but is apparently not affected by removal of the cortex. This result agrees with those of all other experiments.

Comparing YA and YB we see that YA which is living is increased by removal of the living cortex just like ZA was increased in procedures I and II above. On the other hand YB, which is dead, is not at all affected by removal of its dead cortex. Evidently ZA is comparable to YA since both include the living region and are increased by an equal amount. We may perhaps infer from this that YA and ZA measure the E.M.F. of approximately the same region, namely that near A. On the other hand the contacts X and Y with respect to B are not the same, because XB includes the living wood end and is therefore affected by removal of the living cortex, while YB does not include any living wood and is not affected by removal of either living or dead cortex. This is the reason why removal of the cortex does affect XB but does not affect YB. The E.M.F. of XB is decreased in a similar way to that in procedures I and II. However its polarity is finally inverted so that X becomes electronegative to B.

Removal of the living cortex decreases very much (= inverts) the longitudinal wood E.M.F., AB. This is fundamentally the same result as that obtained in all experiments of procedures I and II above. With reference to figure 6, the interpretations of the effects of removal of the living cortex in procedure III are identical with the interpretations given above for procedures I and II, and therefore we shall not repeat them.

PROCEDURE IV

Figure 1.IV, curves, figure 5

EFFECT OF KILLING THE APICAL HALF OF THE INTERNODE BY HEAT.—The experimental procedure was the same as that in the previous experiment except that the third internode of the same tree was used, and the apical half of the internode was killed by immersion in boiling water for four minutes. The times of removal of the dead and living cortex are indicated by the vertical arrows on the curves.

¹ Any partial critic of the oxidation-reduction theory of the origin of continuous bioelectric currents might obviously consider these facts as support for the common theories of bioelectric currents, according to which bioelectric E.M.F.'s are due to differences in concentration of inorganic ions. In this connection the writer merely wishes to repeat that several types of electric potentials in all probability enter into various bioelectric phenomena. The problem which concerns us is the identification of the particular kinds of E.M.F. which occur in different electrical phenomena inherent in the living cell. In any case the existence of a P.D. in a dead system is obviously no secure basis for inferences regarding the nature of the E.M.F.'s in the previous living state of that system.

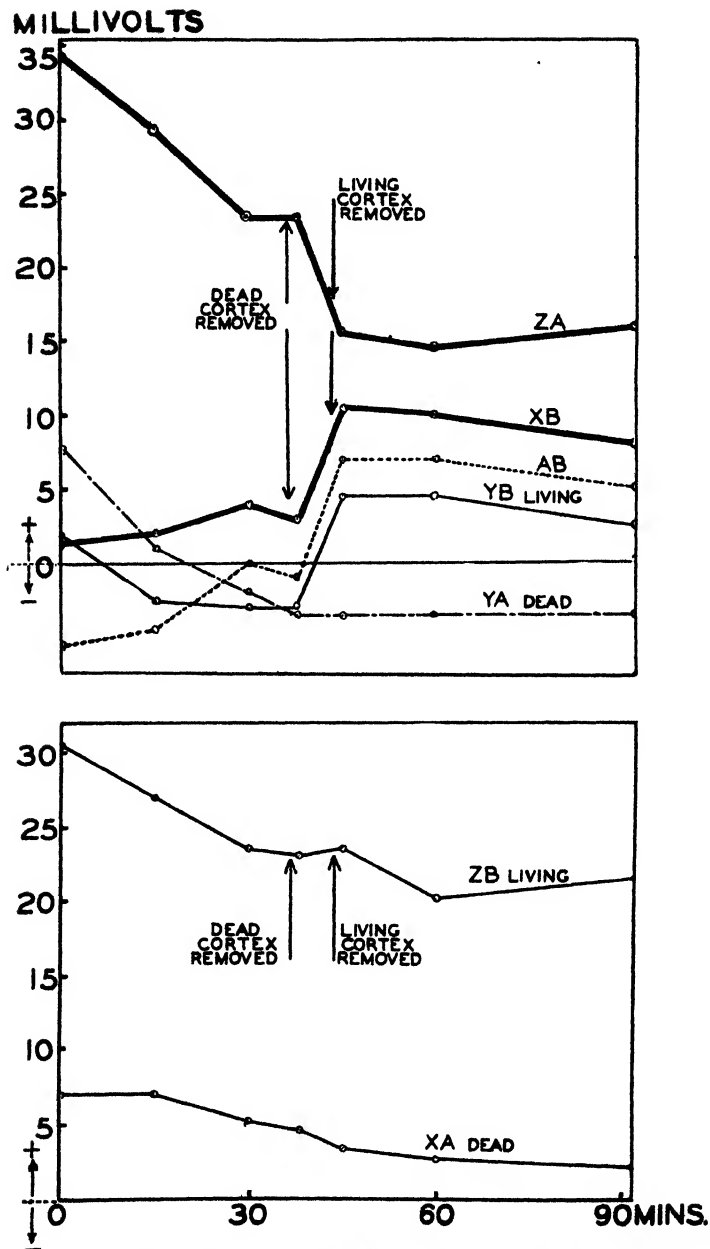


Fig. 5. Upper curves show the changes in E.M.F. produced by removal of apical (dead) and basal (living) cortex between the different pairs of contacts in figure 1.IV. The corresponding curves in figures 4 and 5 should be compared. For interpretation see text.

Again the radial E.M.F.'s, ZB and XA do not show any appreciable affect of removal of the cortex. The longitudinal wood E.M.F., AB, now shows a distinct increase in E.M.F. as compared to a *decrease* in all the previous types of experiment when the living cortex was removed. The question is, why do we obtain opposite effects on AB in the two procedures III and IV when the living cortex is removed?

Referring to figure 6 we recall that the *resultant* of the E.M.F.'s in the cortex is oriented in such a manner that it *augments* (= increases) the radial E.M.F. of the wood in the region d, but *opposes* the radial E.M.F. of the wood in the region c. *After* the removal of the living cortex in procedure III, A is electronegative to B (inverted polarity). *In this case* the resultant E.M.F. of the cortex is evidently opposed to the inherent longitudinal E.M.F. of the wood when the cortex is *in situ*. The result is that the inherent (inverted) longitudinal E.M.F. in the wood between A and B in curve AB of figure 4 is diminished by presence of the cortex and increased by its removal.

The opposite explanation evidently applies to curve AB in procedure IV because the curve shows that *after* removal of the living cortex, A is electropositive to B. In this case the orientation of the resultant E.M.F. in the cortex was again opposite to that in the wood, since removal of the living cortex in procedure IV resulted in an increase in the longitudinal E.M.F. of the wood. Since contacts Z and A of procedure III are comparable respectively to contacts X and B of procedure IV, in their relation to dead and living parts of the stem, we should expect that removal of the living cortex would affect both of these E.M.F.'s in the same manner. The same statement applies to curve ZA of figure 5 and curve XB of figure 4. Both of these expectations are fulfilled as shown by the curves.

Again since the contacts Y and A in procedure III are comparable respectively to contacts Y and B in procedure IV in their relation to dead and living parts, we should perhaps expect that removal of the living cortex would effect both of these E.M.F.'s alike. The curves show that both of these E.M.F.'s are increased. Finally curve YA shows that the E.M.F. of this dead segment is not affected by removal of the dead cortex. This result is the same as that in curve YB of figure 4, procedure III.

Discussion

INCREASE IN THE RADIAL E.M.F. IN THE CORTEX TOWARD THE APEX

The preceding experiments, especially those of procedures I and II, have shown that the effects of removal of the cortex² lead to the conclusion

² By cortex is of course meant the living parts without specifying which particular cells are primarily concerned in the electric phenomena. Identification of the particular cells concerned is of course a problem for the future.

that the resultant E.M.F. of the cortex augments the resultant longitudinal E.M.F. of the wood. This leads to the inference that the radial E.M.F. in an apical part of the cortex is relatively greater than that in a more basal part of the cortex, at least under conditions of absence of injury and stimulation. The arbitrary numbers 5 and 2 in figure 6 are intended to indicate this fact.

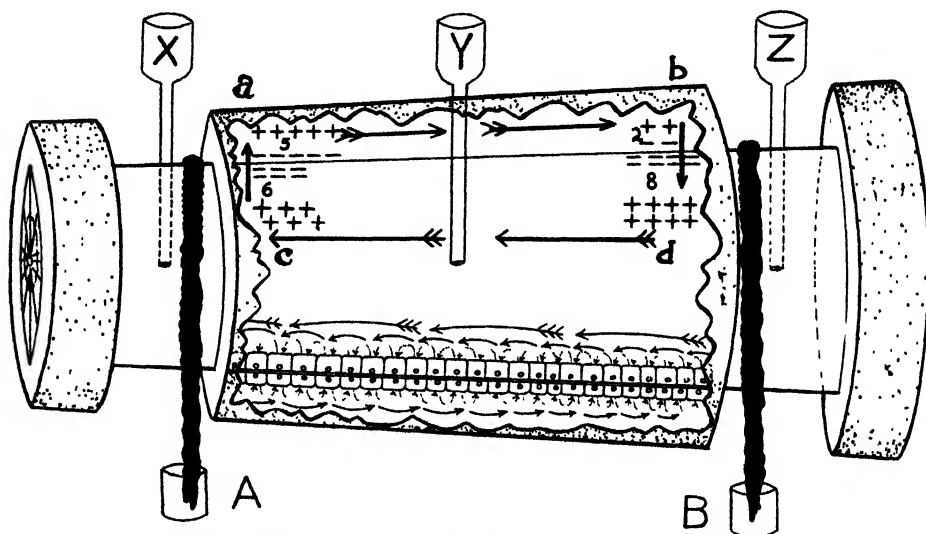


FIG. 6. Diagram of an internode having the same contacts as that shown in fig. 1, procedure II. The numbers 2 and 5 represent arbitrarily chosen values of the radial E.M.F.'s in the cortex at this level, and correspond to the numbers of + and - signs. Similarly the numbers 8 and 6 represent arbitrarily chosen values of the radial E.M.F.'s in the wood. The indicated orientation and relative magnitudes of the radial E.M.F.'s in cortex and wood are those which would fulfill all the requirements of the observed experimental facts. The direction of flow of the resulting current produced by the two systems of E.M.F.'s in cortex and wood is indicated by the direction of the arrows. Note that the resultant E.M.F. in the cortex is in series with that in the wood. At the bottom of the figure is shown two layers of cells in section; these represent diagrammatically the bipolar cambium and other living cells in cortex and wood in which the E.M.F.'s originate. The direction of the arrows indicate the general direction of the flow of electric current. For fuller description see text.

Now if the reader will refer to the diagram E in figure 7, page 271, and table 4, page 275 in the article by LUND (3), it will be found that the magnitude of the basal-apical oblique E.M.F.'s have always the following relation $E_3 > E_2 > E_1$. A specific illustration is given in table 4 where $E_3 = 67.5$ m.v., $E_2 = 52.0$ m.v., and $E_1 = 31.5$ m.v. As the external contact in such a series of measurements is moved farther toward the apex the E.M.F.'s decrease until they finally become zero. Above this zero point the E.M.F.'s

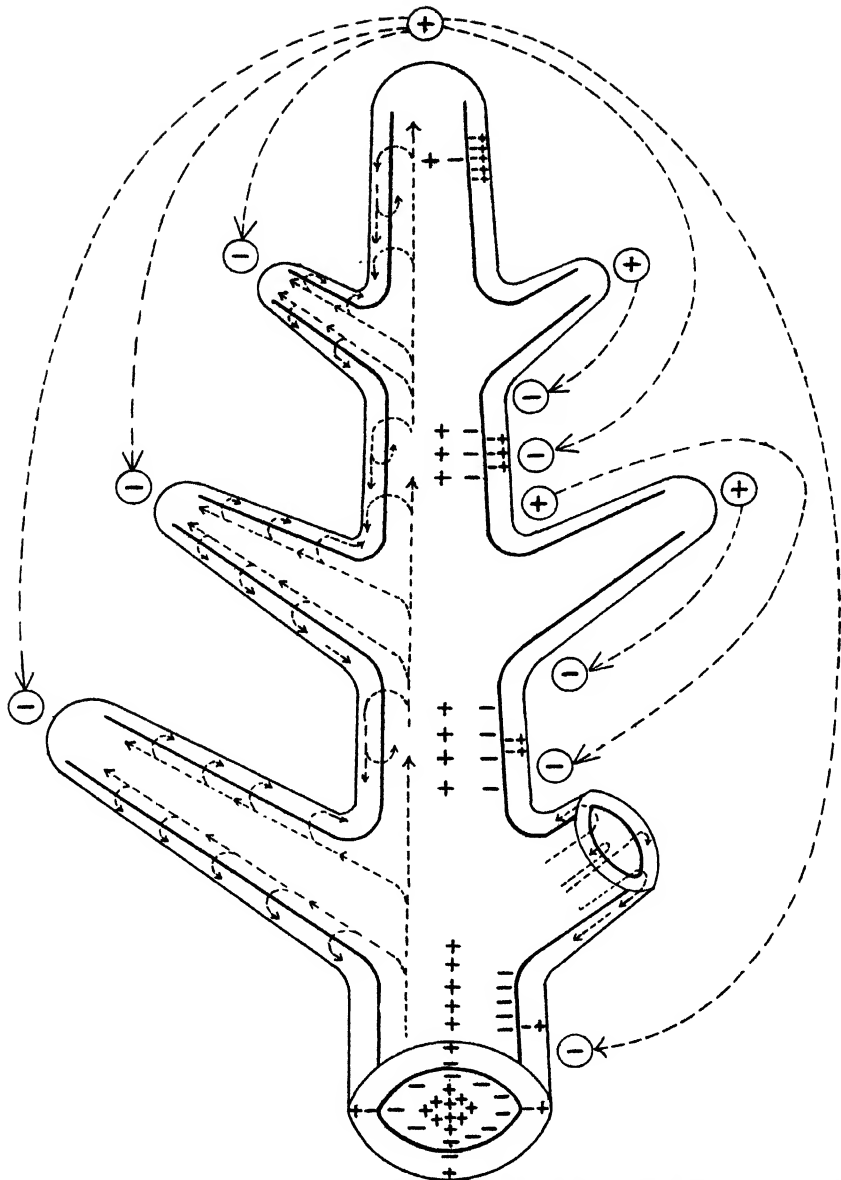


Fig. 7. This diagram attempts to show the approximate distribution and usual orientation of the E.M.F.'s in the wood and cortex of the Douglas fir together with the resulting orientation of the externally observed polarities in the main axis and branches. The relative magnitude of the E.M.F.'s in wood and cortex of the main axis are indicated by the groups of + and - signs on the right hand side of the diagram. Each branch is the seat of a similar distribution of E.M.F.'s.

The system of arrows in the wood and cortex on the left hand side indicates the direction of the resultant E.M.F. in the wood-cortex system in the tree under conditions of absence of stimulation. Experiments show that the E.M.F.'s in the apical regions are especially subject to large fluctuations in magnitude and direction. The diagram is of course not to be taken too literally, although it fits all the observed experimental facts which are known at present.

increase again but the outside apical contact now becomes electropositive (in the external circuit) instead of electronegative to the lower contact in the center of the wood axis.

A series of measurements was carried out on the apical ends of lateral branches. The contacts were those designated by GA, GC, GB, GD, GE and GF in figure 1 of the preceding paper (LUND 5). All of these measurements showed that the apical contacts were electropositive to G except F, and GF exhibited only a small E.M.F. This is interpreted to mean that the total radial E.M.F. of the wood plus cortex diminishes toward the apex. This decrease is due to the decrease in diameter of the wood axis toward the apex, because it has been shown that the radial E.M.F. is proportional to the thickness of the peripheral living active part of the wood axis.

The basal-apical oblique E.M.F.'s in apices are relatively large. The greater part of this E.M.F. must have its origin in the cortex since the orientation of the E.M.F. between these contacts is reversed in the extreme apical regions. In other words the radial and therefore the resultant longitudinal E.M.F. in the cortex of the growing apical segment is greater than that in the thin tubular wood axis. It is evident that in the apex it is the E.M.F. of the cortex which dominates the electromotive phenomena.

In figure 7 this basal-apical increase in radial E.M.F. in the cortex is indicated by the larger number of + and - signs. We conclude from the preceding facts that there must exist a point at some distance below the apex where the radial E.M.F. of the wood plus cortex equals zero.³ The facts taken as a whole show that the radial E.M.F. of the apex is subject to unusually large fluctuations in magnitude when compared to other regions of the tree. The person who likes to contemplate the adaptive features of biological mechanisms will find interesting material in the operation of the mechanism of electric correlation.

GENERAL CONSIDERATION OF THE RESULTS AND THEIR BEARING ON ENERGY TRANSFORMATION IN THE PLANT

The experiments have revealed a group of new phenomena, the consequences of which are of special interest for cell correlation and related processes. In the first place they show in an unequivocal manner that the E.M.F. which is observed when we measure the external electric polarity of the stem and branches of the tree is the algebraic result of a system of internal E.M.F.'s, the elements of which are located in two main regions, namely the cortex and wood. The experiments show that the *resultants* of these systems of E.M.F.'s augment one another, that is to say they operate

³ This point corresponds to that on the main axis of the stem at which the extrapolated curves of E.M.F. in LUND ((3) figure 4, page 265) cuts the horizontal axis.

in series like two complex systems of batteries. In general they show that an electric current flows downward in the outer cortex and upward in the wood axis. The results therefore confirm previous observations but in addition show that the cortex is the seat of a separate E.M.F. Figure 7 is presented as an approximate summary of the facts to date. To help visualize a little more accurately the direction of flow of the transverse and longitudinal components of the currents, the bipolar cambium and other bipolar origins of the E.M.F. are represented diagrammatically in figure 6 by two layers of cells with a system of arrows to indicate the general direction of the correlation currents. Future experimental analysis of the distribution of correlation currents will of course be toward a description of the patterns in microscopic dimensions. In all probability these patterns will be found to correspond more or less to the complex cellular architecture of the wood and cortex in which they originate and in which their energy is being transformed and dissipated.

One fact of extreme interest which must be clearly recognized is that when we measure the E.M.F. between any two contacts the magnitude of this E.M.F. is in general not necessarily directly proportional to the total electric current which flows in the local circuits in the tissues between the electrode contacts. In other words the system operates more like a complex network of conductors with a relatively fixed pattern of local origins of E.M.F.'s. It appears certain from this consideration that relatively large amounts of electrical energy are being transformed into electrical work and heat, which would not be indicated by single measurements of E.M.F.'s or electric currents led off from a single pair of contacts as we do in actual practice.

It is not improbable that a considerable part of the total heat energy output by the plant is derived from continuous electric currents, the energy of which appears only secondarily as heat. If this is true then it is obvious that the experiments have opened to us a new vista of the phenomena of energy transformations in the plant. We shall discuss briefly some of the possibilities in what follows.

CONTINUOUS CORRELATION CURRENTS AS THE POSSIBLE SOURCE OF ENERGY FOR ELECTROENDOSMOTIC TRANSPORT IN THE PLANT

The writer can not forego the opportunity at this time of presenting what appears to him a distinct possibility, namely that one of the functions of the continuous electric current which is directed upward in the wood is to supply electrical energy for electroendosmotic flow of sap in an upward direction in the conducting vessels of the wood. This suggestion may also apply to a downward flow in the cortex as well as transport across the stem.

If this is the case we would expect that the well known equation for velocity of flow in a system of capillaries caused by the application of an E.M.F. would apply, namely,

$$v = \frac{q \epsilon H \zeta}{4 \pi \eta l}$$

In this equation v = volume flow, q = area of cross-section of the capillary system, H = the applied E.M.F., ϵ = the dielectric constant of the liquid, η = the viscosity, and l = length of the capillary system, ζ = the electrokinetic potential between the liquid and wall of the capillary. Suppose we consider a certain bundle of conducting vessels or a single conducting vessel in the water conducting region of the wood (OVERTON 9). The orientation of the radial and resultant longitudinal E.M.F.'s is that represented in the diagram figure 6. It is evident that q , ϵ , η and l are determinable quantities. H is supplied by the inherent E.M.F., leaving the electrokinetic potential ζ to be considered. Now it has been shown by STAMM (10) that the orientation of the electrokinetic potential of the walls of wood vessels against water is such that the liquid in all the types of wood which he examined namely, Sitka spruce, Alaska cedar, western red cedar, western hemlock, Douglas fir and yellow poplar, moved from anode to cathode, showing definitely that water with respect to the capillary vessel wall carries a positive charge.

STAMM's experiments therefore demonstrate first, that an electrokinetic potential exists between the water and the wall of the conducting vessel. The presence of the electrokinetic potential as the equation shows, is a *sine qua non* for the occurrence of electrokinetic flow, for if ζ is 0 then $v = 0$. The second significant fact about STAMM's experiments is that the electrokinetic potential is oriented in the right direction, that is, water is electropositive to the wall of the vessel and therefore the observed inherent E.M.F. in the wood, figure 6, which now takes the place of H in the equation will of necessity tend to transport the water upward and not downward in the vessels of the wood.

With these facts in mind a few preliminary experiments were carried out on the apexes of lateral branches of the Douglas fir. The procedure was as follows. The apical part of a lateral branch was cut off about one inch below the origin of the two most distal symmetrically placed lateral shoots. Care was taken to select tips of branches in which these two lateral shoots were placed exactly opposite one another at the base of the main axis shoot. The cut basal end was dipped into a solution of eosin in water. Tap water electrode contacts were attached to the tips of the two lateral shoots. A constant electric current of a few microamperes was sent into the apex of one of the symmetrical lateral shoots and out through

that of the other. The applied current therefore passed downward in one and upward in the other shoot. The current was left on usually for eight to twelve hours. During this period the solution usually ascended several cm. in the wood axis.

Removal of the cortex showed that in most cases the distance to which the dye had risen in the two lateral shoots was unequal. In all the experiments showing distinct inequality the solution had penetrated to the greatest distance in that lateral shoot in which the electric current flowed from base to apex in the wood. In some of these preliminary experiments the difference was very marked while in others the result remained uncertain.

The results of these preliminary experiments are merely to be considered as suggestive. However, the results indicate agreement with the interpretation which we have presented above.

At present the phenomena are being investigated with a fuller consideration of the many factors which are involved. It is obvious that such phenomena as guttation and bleeding pressures may be the expression of the same type of mechanism.

The possible application of correlation potentials in roots (LUND and KENYON, 6) to the problem of differential absorption of ions will be considered in later papers.

Summary

1. The results of the experiments constitute direct and conclusive evidence that the principle of summation of E.M.F.'s applies to phenomena of electric correlation between living cells in the Douglas fir.

2. The cortex of the Douglas fir is the origin of a characteristic E.M.F. The orientation of the radial E.M.F. in the cortex is opposite to that in the wood.

3. Several lines of evidence show that this radial E.M.F. in the cortex increases toward the apex, while that in the wood decreases.

4. It is shown that the resultants of each one of the two systems of E.M.F.'s in the cortex and wood operate as if they are placed in series.

5. Removal of a ring of living cortex which lies between two contacts placed on or in the wood axis results in a permanent change in the previously observed E.M.F. between these contacts. The direction of change depends upon the position of the contacts.

6. Removal of similar rings of cortex from stems which had been killed by heat did not produce any such marked effects.

7. Electromotive forces are present in stems killed by heat, but the behavior of such E.M.F.'s is radically different from those in the living cortex.

8. Attention is called to the possible rôle under certain conditions of electric correlation currents in the wood as a source of energy for electroendosmotic transport of water upward in the conducting vessels of the wood. It is shown that all present known facts agree with this possibility.

9. The observed small quantities of electric energy which may be led off from two contacts on the tree are not to be considered as indicative of the actual amounts of electrical energy which are transformed into work and heat in the plant. There is every reason to believe that the quantity of electrical energy which is transformed is much larger than has heretofore been suspected.

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NOTES ON THE NUTRIENT REQUIREMENTS AND THE HISTOLOGY OF THE CRANBERRY (*VACCINIUM MACROCARPON* AIT.) WITH SPECIAL REFERENCE TO MYCORRHIZA¹

RUTH M. ADDOMS AND F. C. MOUNCE

(WITH THREE FIGURES AND TWO PLATES)

Introduction

The nutrition of the cranberry, as well as of various other members of the Ericaceae, is complicated by the fact that under ordinary cultural conditions the green plant exists in close association with a fungus. Although an association of a fungus with the roots of a higher plant is called a mycorrhiza, the literal definition of the word must be extended somewhat to include the condition that exists in the Ericaceae, for in this family the fungus is not confined to the roots, but is coextensive with the growth of the plant, occurring in practically every organ. The fungi associated with the Ericaceae were named by TERNETZ (14) in 1907 as strains of *Phoma radialis*, and have since been isolated by RAYNER (9) and other workers. The most complete summary of the work that was done on mycorrhiza by various workers prior to 1927 is to be found in a monograph by RAYNER (10).

Field experiments by various workers (1, 12, 15) have led to conflicting reports in regard to the value of various nitrogenous fertilizers. It is possible that the presence of the endophytic fungus may make available forms of nitrogen that could not otherwise be utilized by the green plant. The present project was undertaken in an attempt to contribute to our knowledge of the fertilizer requirements of the cranberry, and of the structural and nutritional relationships between the cranberry and its endophyte. This paper represents only a preliminary report; it is hoped that a continuation of the work will yield further results.

Methods

CULTURAL

All of the plants used in these experiments were of the variety Early Black.² On April 26, 1930, several hundred rooted cuttings selected for uniformity were removed from bog soil, washed clean, transplanted to washed quartz sand in new eight-inch flower pots, two to four plants to a

¹ Journal Series paper of the New Jersey Agricultural Experiment Station.

² The plants were obtained through the courtesy of MISS ELIZABETH C. WHITE, of Whitesbog, N. J.

pot, and placed in a greenhouse. The stems of these plants (fig. 1) averaged from 13 to 15 cm. in length. For a period of three weeks, during which time they resumed growth after transplantation, they received a full nutri-



FIG. 1. Rooted cuttings, as transplanted to sand culture on April 26, 1930. $\times \frac{1}{4}$.

ent solution (see Series 2, below). On May 17 they were divided into 4 series, which from then until the latter part of August received the following nutrient treatments:

SERIES 1.—21 pots formed a culture triangle, each pot receiving a different proportion of MgSO_4 , KH_2PO_4 , and $\text{Ca}(\text{NO}_3)_2$ as shown in table I, but all having the same total osmotic concentration (6). The salts were supplied by SHIVE's constant-drip method (13). This series was run in duplicate.

SERIES 2.—28 pots received solution R_2S_4 of the above triangle, a solution which had been shown by previous experiments to be well-suited to the growth of many kinds of plants. Nitrogen was supplied as $\text{Ca}(\text{NO}_3)_2$.

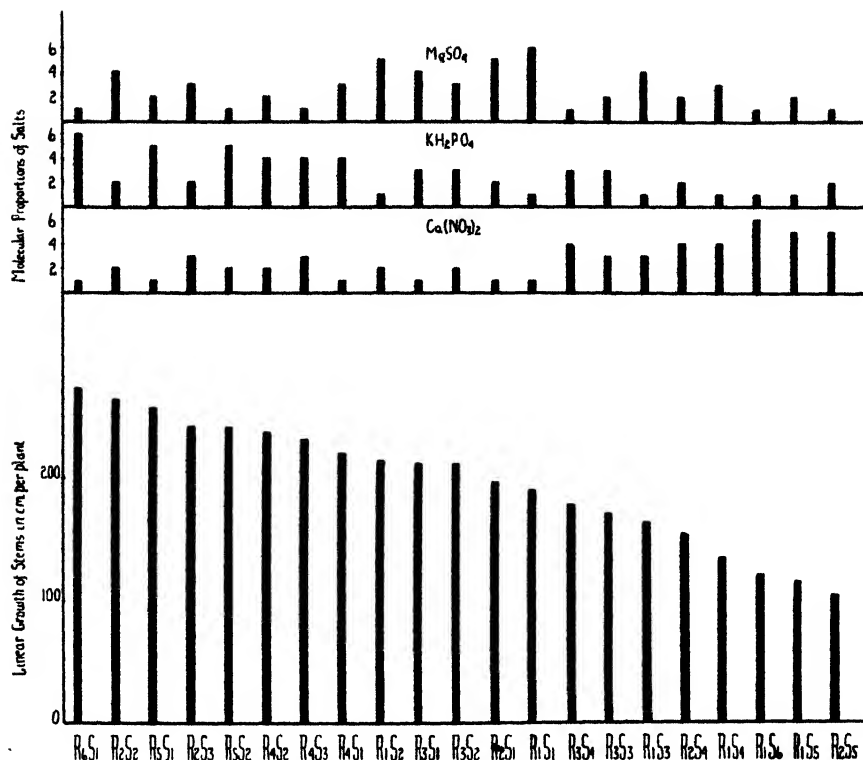


FIG. 2. Below, block diagram showing the amount of linear growth of stems in each culture of the triangle. Above, block diagrams showing the molecular proportions of $MgSO_4$, KH_2PO_4 , and $Ca(NO_3)_2$, respectively, in each of the nutrient solutions of the triangle.

SERIES 3.—28 pots received a solution entirely lacking in nitrogen. Solution R₂S₄ was modified by the substitution of $CaCl_2$ for $Ca(NO_3)_2$. From time to time the sand was tested for nitrates, always with negative results.

SERIES 4.—6 pots received a solution of the following composition (5) in which nitrogen was supplied as ammonium sulphate:

SALTS USED	PARTIAL VOLUME-MOLECULAR CONCENTRATION
$(NH_4)_2SO_4$	0.0014
KH_2PO_4	0.0020
$CaCl_2$	0.0073
$MgSO_4$	0.0071

TABLE I
COMPOSITION OF CULTURE SOLUTIONS USED

SOLUTION NUMBER	MOLECULAR PROPORTIONS			PARTIAL VOLUME-MOLECULAR CONCENTRATIONS			AVERAGE STEM GROWTH PER PLANT
	KH_2PO_4	$\text{Ca}(\text{NO}_3)_2$	MgSO_4	KH_2PO_4	$\text{Ca}(\text{NO}_3)_2$	MgSO_4	
							<i>cm.</i>
1R1S1	1	1	6	0.0027	0.0027	0.6161	190
S2	1	2	5	0.0025	0.0049	0.0123	213
S3	1	3	4	0.0024	0.0071	0.0094	163
S4	1	4	3	0.0022	0.0089	0.0067	136
S5	1	5	2	0.0022	0.0108	0.0043	116
S6	1	6	1	0.0020	0.0122	0.0020	121
R2S1	2	1	5	0.0053	0.0027	0.0132	197
S2	2	2	4	0.0049	0.0049	0.0099	263
S3	2	3	3	0.0047	0.0071	0.0071	242
S4	2	4	2	0.0045	0.0090	0.0045	155
S5	2	5	1	0.0041	0.0104	0.0021	103
R3S1	3	1	4	0.0076	0.0025	0.0101	211
S2	3	2	3	0.0072	0.0048	0.0072	211
S3	3	3	2	0.0068	0.0068	0.0045	171
S4	3	4	1	0.0065	0.0086	0.0021	178
R4S1	4	1	3	0.0099	0.0025	0.0074	220
S2	4	2	2	0.0094	0.0047	0.0047	238
S3	4	3	1	0.0090	0.0068	0.0022	231
R5S1	5	1	2	0.0123	0.0024	0.0049	257
S2	5	2	1	0.0118	0.0047	0.0023	241
R6S1	6	1	1	0.0145	0.0024	0.0024	272

This series was started about four weeks later than the others.

In all of the cultures the total osmotic concentration was approximately one atmosphere. Iron was supplied from time to time as ferrous sulphate. The acidity of the various nutrient solutions ranged from pH 4.9 to pH 5.6, a range somewhat less acid than that of most cranberry bogs.

Supplementary experiments, carried on from time to time as need arose, are described later in this paper.

HISTOLOGICAL AND MICROCHEMICAL

Cranberry plants from the cultures described above, and also plants of the same variety from a commercial bog were examined microscopically for details of structure of the cranberry plant and of its endophytic fungus.

Microchemical tests were in general those suggested by Dr. ECKERSON (3) of the Boyce-Thompson Institute. For the most part it was found desirable to study fresh material, colored by means of ruthenium red, aniline blue, mercurochrome, or other stains. For fixed material both orange G and safranin were found useful when sufficiently dilute.

Results

CULTURAL

All of the plants, with the exception of those that received no nitrogen, and the three or four poorest cultures of the triangle, were fairly green, grew vigorously, and produced runner-growth greatly exceeding that of similar plants that were grown for the same period in a commercial cranberry bog. In fact, the plants would be regarded by a commercial cranberry grower as too vegetative for the production of fruit. The linear growth of stems of the various cultures in the triangle is shown in table I as average growth per plant in centimeters. Correlation between amount of stem-growth and composition of nutrient solution is shown graphically in fig. 2. The linear growth of stems in Series 2 to 4 is given below:

SERIES	LINEAR GROWTH OF STEMS (AVERAGE OF 4 PLANTS) IN CM. PER PLANT
Series 2, nitrate (R_2S_4)	227
" 3, minus nitrogen	52
" 4, ammonium	370

Fig. 3 shows the general appearance of representative plants on August 22, and gives indication of the vigor of their growth. With the exception of the minus-nitrogen series and of the plants that received the highest concentrations of nitrate, all plants produced large and apparently healthy root systems.

Bog soil, the usual habitat of cranberry plants, has a high content of humus and a relatively low content of nitrates. Because of these facts, an additional experiment was conducted. Five grams of dried blood, which is often used as a fertilizer in cranberry bogs, and which has a high content of organic nitrogen, were added to each of two pots of cranberry plants that were receiving the minus-nitrogen solution (Series 3). The plants responded quickly to the treatment, developed in apparently healthy manner, and produced runner-growth comparable to that of the ammonium series (Series 4). After these cultures had been continued for several weeks, the sand was tested for nitrates, ammonia, and amino acids. The diphenyl-

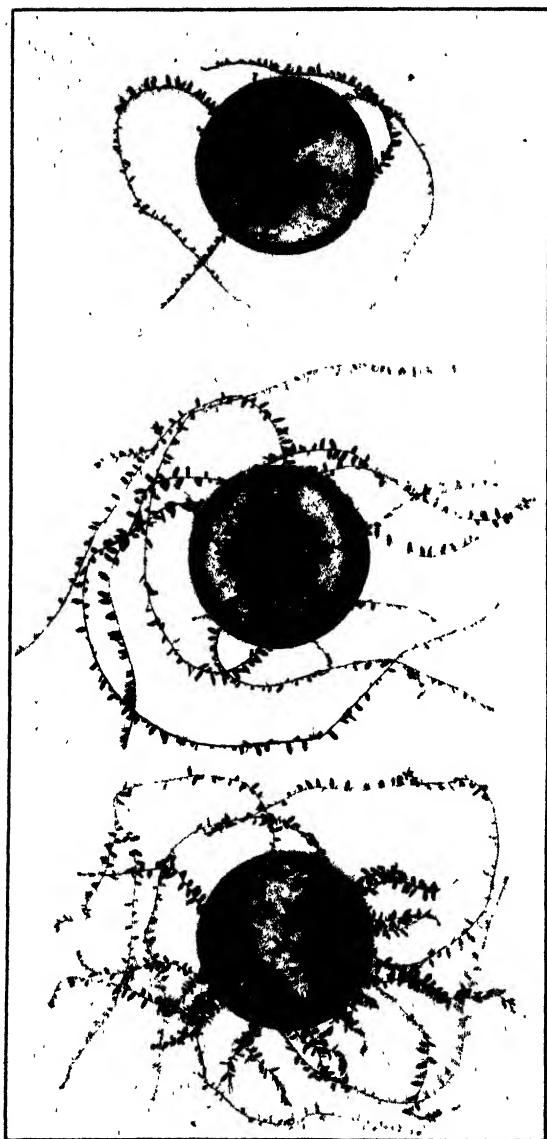


FIG. 3. Representative plants as they appeared on August 22, 1930. Top, culture R_1S_2 (high nitrate). Middle, culture R_2S_2 (low nitrate). Bottom, ammonium culture. $\times 1/10$.

amine test indicated the presence of only a faint trace of nitrates; aspiration yielded ammonia; SORENSSEN's formal titration method showed a considerable quantity of amino acids.

It thus seems possible, although nothing has been proved, that the cranberry plant or its endophyte or the combination of the two (mycorrhiza) can utilize amino acids. Additional evidence is furnished by the results of an experiment performed during the latter part of August. Three pots of minus-nitrogen plants (Series 3) were selected for uniformity; one was supplied with a solution containing nitrogen in the form of nitrate only (solution R_2S_4), one in the form of ammonium (solution of Series 4), and one in the form of amino acids only (glycine, leucine, tyrosine, and asparagine in a total concentration of 0.1 per cent.). All solutions were supplied by SHIVE's constant-drip method. The amino acid solution was boiled each day before it was added to the cultures, to minimize bacterial action. The sand was tested frequently for nitrates, always with negative results; it did, however, contain a trace of ammonia. The plants receiving ammonium and amino nitrogen respectively responded much more quickly than those receiving nitrate nitrogen, and within a week showed appreciable growth.

HISTOLOGICAL AND MICROCHEMICAL

The roots of the cranberry plant, in common with those of many other members of the Ericaceae, are very fine and much branched, with no root hairs, so that nutrients must be absorbed by unelongated epidermal cells or must enter those cells through the mycelium with which they are infested. Mycorrhiza was observed in all the culture series of these experiments as well as in plants that grew for the same period in a bog. The bog plants, however, were much more heavily infected than any of the sand cultures. The minus-nitrogen series in general showed least infection, although a few individual plants in this series were heavily infected.

The mycelium forms a branching mass over the surface of the young roots (plate XI, fig. 4), extending practically to the tip. Young hyphae are slender and colorless, older ones larger and brown. Branches of the mycelium penetrate epidermal cells, and within these cells form intertwined masses, as shown in plate XI, fig. 5.

In general the root is similar to that described by JANSE (4) for other species of *Vaccinium*. The internal structure is most readily observed in cross-section (plate XII, fig. 8). Beneath the epidermis, which consists of conspicuously large cells, lies a single layer of cortical parenchyma, and beneath this, the endodermis, which becomes thick-walled and suberized at an early age. The pericycle is one or two cells thick, and the protoxylem points are usually three in number, although there is some variation. My-

celium is plainly visible in the layer of cortical parenchyma, although its form is not so clear in fixed as in fresh material. Partial digestion of the mycelium may occur, resulting in the appearance in fresh material of granular masses rather than of distinct hyphae. Mycelium has not been observed in the endodermis or in the stele of young roots; this finding is in accordance with that of RAYNER (10) for *Calluna*. In older woody roots, however, it has been observed by both Dr. ECKERSON and the present authors in the parenchyma of xylem and phloem.

Stems of the plants that had received different cultural treatments showed greater differences than the roots. The minus-nitrogen (Series 3) plants grew so slowly that a relatively small portion of each stem was succulent, a relatively large portion woody and stored with starch. Such an accumulation of carbohydrates is not peculiar to the cranberry, but represents the usual condition in plants in which vegetative growth is curbed by deficiency of nitrogen or of certain other elements. Also, the plants of Series 1 that received the highest concentrations of nitrate grew slowly and were high in carbohydrates. Otherwise the plants of the various series showed no significant differences in stem structure.

The structure of a portion of a young stem is shown in plate XI, fig. 7. Strands of primary xylem are numerous, and cambial activity results in the formation of a continuous vascular cylinder, interrupted by uniseriate rays. Just outside the sieve-tubes and companion cells of the phloem are several layers of thin-walled parenchyma and several layers of angular cells that gradually lengthen and become fibers, although never extremely thick-walled. These layers of parenchyma and fibers probably represent pericycle, although since an endodermis is not clearly distinguishable there is no demarcation between stele and cortex. The several layers of thin-walled cortical cells, some of which contain an anthocyanin pigment, are bounded by an epidermis that is rich in oil and is heavily cutinized. In the young stem, chloroplasts are located chiefly in cortical parenchyma and in medullary rays. Calcium oxalate crystals are common in cortical parenchyma, especially at the nodes, in the basal portion of axillary buds, and in the apical meristem.

As the stem grows older, the outer regions are split off as a result of the activity of a cork cambium that is formed in the parenchyma directly external to the phloem. Thus the fibers become the outermost layer, and as expansion continues they too are crushed and split off, and their place is occupied by cork. Further increase in diameter is slight, and a cranberry stem always remains relatively slender, although it becomes very tough and woody as lignification of the internal tissues takes place. Pith, xylem, and rays, all become very thick-walled and lignified as shown by their reac-

tion with phloroglucin. The walls are distinctly pitted—pith and ray cells with simple pits, vessels and tracheids with pits of various types. Throughout the summer, cells of the pith, and to some extent those of the rays, were observed to contain large quantities of starch but very little oil.

The leaves remain functional for more than one year, and as they age become somewhat tough and leathery because of the heavy cuticle and the large amount of mechanical tissue associated with the main veins (plate XI, fig. 6). The mesophyll is surprisingly loose, with large intercellular spaces. During the summer both palisade and spongy cells usually contain large quantities of starch, as well as one or two globules of oil per cell. Oil is found in considerable quantity in the epidermis also. Calcium oxalate is conspicuous in terminal and axillary buds; in a mature leaf it is usually localized in the petiole and in the parenchymatous sheath that surrounds the veins. Stomata are confined almost entirely to the lower epidermis.

The infection of shoot tissues of ericaceous plants by the mycorrhizal fungus of these plants was first described by RAYNER (9) for *Calluna vulgaris*, and the discoveries have since been extended by her and several other workers to other plants, until such association of green plant and fungus has come to be regarded as a common condition in Ericaceae, although the details have been worked out for comparatively few plants.

A recent paper by RAYNER (11) describes in detail the distribution of the mycelium in the seed. The present authors have observed it in the ovary wall (plate XII, fig. 11) and in cells of the seed coat, but her more extensive researches have shown that it is present also in the endosperm of the resting seed. In the early stages of germination the tissues of the embryo become infected, and thereafter the infection of shoot tissues is assured. As cranberries are usually propagated by cuttings, mycelium is present in the stems when they are set out.

Study of the endophytic fungus in the shoot tissues is rendered difficult by its extremely small size, and by the fact that it is easily broken and washed away. Moreover, not all parts of all shoots are infected, so that prolonged and careful examination under high magnification may be necessary in order to detect the fungus in its relation to the cranberry plant. In general, fresh sections either unstained or stained lightly with mercurochrome or with ruthenium red, proved superior to microtome sections. Young mycelium was found to stain readily with ruthenium red and with aniline blue, indicating a content of pectic substances and of callose respectively; older mycelium did not take these stains.

In the present experiments, plants from all the various culture series were examined for the presence of the endophytic fungus. It was found in all, but least abundantly in the minus-nitrogen plants (Series 3). In

August the mycelium was usually most easily observed near the tips of rapidly growing runners. It was noted in practically all the different types of cells of the young stem except epidermal cells, but was most conspicuous in parenchymatous cells, especially of the pith (plate XII, fig. 9) and cortex. Usually some of the hyphae appeared to be in close association with the walls, but others extended into the protoplast. In cells that contained much mycelium, little or no starch was present and no chlorophyll. No starch grains were observed in the mycelium, but granules of glycogen were present, and at times globules of oil. Upon the addition of alcohol, crystals that were apparently glycine were also noted in the mycelium. Regardless of the concentration of nitrates in the external solution, no nitrates could be detected at any time in either the cranberry plant or its endophytic fungus.

In April and May the brown-walled mycelium in the older part of the stem was much more easily observed than the colorless mycelium near the rapidly growing tips, but as the season advanced it became less and less conspicuous because of the partial or complete disintegration, as shown in plate XII, fig. 12. Such disintegration or "digestion" has been described and figured by RAYNER (9) for *Calluna*. In old cranberry stems from both cultures and bogs the writers frequently observed dark masses of high stainability, especially in the conductive elements of the xylem and in the rays. In all probability they were phases of digestion of the fungus, for at no time could the writers detect the presence of other fungi such as those that are associated with the various diseases of cranberries. Dark masses, apparently similar to those just mentioned, were noted in leaves also, where they could be observed in greater detail. In leaves, however, they were in the mechanical tissue associated with the larger veins, rather than in the conductive elements of the xylem. Plate XI, fig. 6 and plate XII, fig. 10 show them as they appeared in cross and longitudinal section respectively. The smaller cells seemed to be entirely filled, whereas in the larger cells the masses were more open and apparently vacuolate. During the latter part of the season these dark masses were the only evidence of fungous invasion that could be noted in the leaves.

Discussion

A cranberry plant is thus in intimate association with an endophytic fungus. There are three possible methods of infection: (1) infection of young roots from external mycelium present in the soil, (2) infection of seedlings from tissues of the seeds at the time of germination, and (3) infection of plants propagated by cuttings from mycelium in the tissues of the cutting.

The nutritional physiology of such a complex offers many problems. It is said (2, 8) that *Phoma radicis* is capable of nitrogen-fixation, but the amount is small, and in these experiments proved entirely inadequate to support vigorous growth of the host, as shown by the poor growth of the plants of the minus-nitrogen series (Series 3).

In their natural state cranberries and many other ericaceous plants usually grow in soils that are conspicuously low in nitrates. In contrast to the general belief, the authors found by chemical test that a sample of bog soil may retain nitrates for several months after the addition of nitrate fertilizer. The fact remains, however, that under ordinary growing conditions the available supply of nitrates is very low.

Analysis of fig. 2 indicates that within the nitrate series (Series 1), smallest amounts of vegetative growth were associated with highest concentrations of calcium nitrate. Presumably the high concentration of the nitrate ion was a limiting factor. It is possible that the high concentration of calcium was toxic, but this seems unlikely, in view of the fact that although the minus-nitrogen (Series 3) plants received a relatively high concentration of calcium, they grew luxuriantly as soon as nitrogen in the form of ammonium sulphate was added. It also seems unlikely, from examination of fig. 2, that the poor growth of high-nitrate plants was caused by inadequate concentrations of either of the other two salts.

Although an excess of nitrate was directly or indirectly injurious, smaller amounts promoted growth. Nearly all of the plants of Series 1 exceeded in amount of runner growth similar plants that had grown for the same period in a bog. Moreover, when minus-nitrogen plants were supplied with a nutrient solution containing nitrates they showed appreciable increase in vegetative vigor, although recovery was slow. In these cultures ammonia was not present in the sand in quantities sufficient for detection by macrochemical methods.

As stated above, nitrates could not be detected at any time in either the cranberry plant or its endophytic fungus. An attempt was made therefore to determine whether at low temperatures nitrates might be absorbed but not assimilated. Such a condition is known to exist for asparagus (7). Cranberry plants were kept at temperatures of 2° C., 5° C. and 10° C. respectively for periods ranging from 8 to 48 hours. At no time could nitrates or nitrites be detected microchemically. When the plants were returned to higher temperature they resumed vigorous growth and were apparently uninjured by the treatment. It would thus seem that the absence of nitrates in cranberry plants is not associated with their rapid assimilation within the plant. In a consideration of this problem, the results of Dr. ECKERSON's experiments on reductase activity are significant.

Throughout the period of active growth, she made tests of various parts of plants, from both sand cultures and bogs. The extracted juice of even the finest roots contained only faint traces of material capable of reducing nitrates to nitrites. In a sample of older plants that were heavily infected with the endophyte, a trace of reductase activity could be detected. Results to date are too fragmentary to justify any conclusions as to the changes that take place between the initial absorption of nitrates, if such occurs, and the subsequent growth of new tissues.

It is significant that in amount of vegetative growth, plants of the ammonium series (Series 4) far exceeded those of the two nitrate series (Series 1 and 2), although they were started four weeks later. The intake of ammonium and its rapid passage through the plant were followed by means of Nessler's reagent and by the formation of crystals of ammonium chloroplatinate. Results of the present experiments suggest the ammonium sulphate in itself is not toxic to cranberry plants, although several field trials in New Jersey (1, 12) have indicated that under certain conditions it may not be used successfully as a fertilizer in cranberry bogs. Before definite conclusions can be reached, further experiments should be conducted with several different ammonium salts in several different concentrations, in cultures of several different degrees of acidity, and the results of these experiments should be correlated with those of further field experiments.

In the experiments described on page 657 it was found that dried blood, a commercial fertilizer that has a high content of organic nitrogen, stimulated practically as much vegetative growth as did ammonium sulphate in the concentration employed. Although it is impossible to state what changes took place between the time that organic nitrogen was applied to the culture and the time that nitrogenous compounds were absorbed by the plant or the mycorrhiza, it is interesting to note that analysis of sand from these dried blood cultures showed the presence of appreciable amounts of ammonium and amino nitrogen, but only traces of nitrates. It should also be noted that ammonia was found to be present in the sand of the cultures to which amino acids had been added.

It would thus seem from these preliminary experiments that ammonium sulphate can be used to promote vegetative growth of cranberry plants. Further experiments may show that the growth responses are modified by variations in acidity and other environmental factors. To what extent the green plant is aided by the endophytic fungus in the absorption and utilization of nitrogen has not yet been determined.

Summary

1. Cranberry plants were grown for several months in sand cultures supplied with nutrient solutions containing nitrogen in the form of nitrate

and ammonium respectively, and with a nutrient solution lacking in all forms of nitrogen. All except the minus-nitrogen series produced runner-growth exceeding that of similar plants grown in a bog.

2. Plants of the ammonium series produced noticeably greater runner-growth than those of the nitrate series.

3. Low concentrations of nitrate promoted vegetative growth, but high concentrations produced little growth under the conditions of these experiments.

4. Mycorrhiza was found in the cultures of all series, but least in the minus-nitrogen series.

5. The small amount of vegetative growth in the minus-nitrogen series indicated that if nitrogen-fixation by the endophyte (*Phoma radicis*) occurred, it was quite inadequate as a source of nitrogen for the cranberry plant.

6. Mycelium of the endophyte was found throughout the stem system of the plant, including fruits and seeds.

7. The mycelium forms a branching mass over the surface of the cranberry roots, which are very small and lack root hairs. Hyphae penetrate the epidermis and cortical parenchyma, and form mycelium in the cells.

8. In the stem, mycelium is most abundant in parenchymatous cells, especially of pith and cortex. It may be confined chiefly to the walls or it may be found throughout the protoplast. Oil and glycogen are often present in the fungus.

9. Nitrates could not be detected in either cranberry plant or endophyte at any time.

10. Experiments with organic nitrogen strengthen the conclusion that ammonium nitrogen can be utilized by the cranberry plant. The extent to which the endophytic fungus is concerned in the nitrogen-metabolism of the cranberry plant has not yet been determined.

The authors wish to acknowledge with sincere gratitude the counsel and active help of Dr. G. T. NIGHTINGALE throughout the progress of the experiments.

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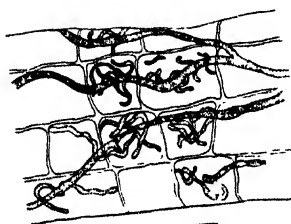
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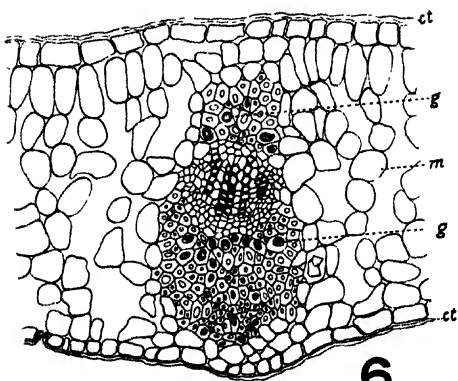
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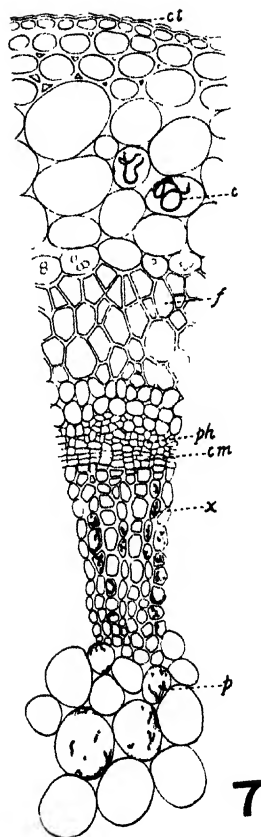
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EXPLANATION OF PLATES

All drawings were made with the aid of projection apparatus, from fresh material except as otherwise noted.

PLATE XI

FIG. 4. External view of portion of root, showing mycelium on the surface. $\times 106$.

FIG. 5. Portion of root, showing mycelium. Large hyphae are on the surface of the root, small ones inside the epidermal cells. $\times 480$.

FIG. 6. Cross section of portion of leaf.

ct = cuticle

g = granular material in cells of mechanical tissue

m = spongy mesophyll

This drawing was made from a prepared slide. $\times 180$.

FIG. 7. Cross-section of portion of young stem. $\times 180$.

ct = cuticle

c = mycelium in cell of cortical parenchyma

f = fiber

ph = phloem

cm = cambium

x = mycelium in xylem

p = mycelium in cell of pith

PLATE XII

FIG. 8. Cross-section of young root.

ep = mycelium in epidermal cell

en = endodermis

c = mycelium in cell of cortical parenchyma

px = protoxylem point

This drawing was made from a prepared slide. $\times 180$.

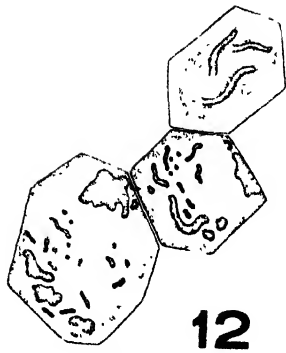
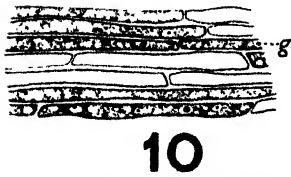
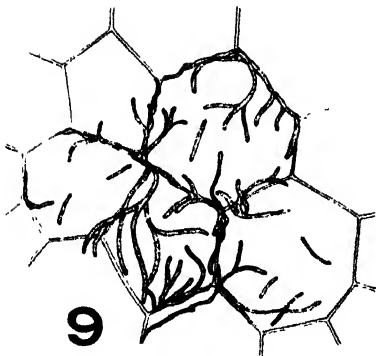
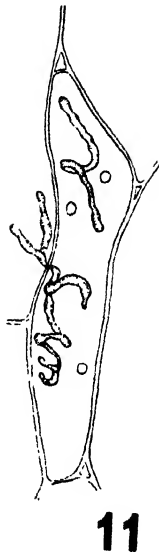
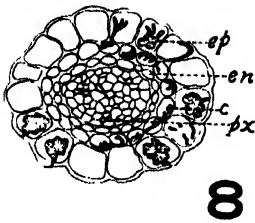
FIG. 9. Mycelium in cells of the pith of a young stem. $\times 480$.

FIG. 10. Cells of the mechanical tissue of a vein of a leaf, in longitudinal section.

g = granular material

FIG. 11. Mycelium in cells of the ovary wall. $\times 480$.

FIG. 12. Cells from the pith of a stem, showing stages in disintegration of the mycelium.
 $\times 480$.



ADDOMS AND MOUNCE: NOTES ON CRANBERRY

EFFECT OF CUTTING AND FERTILIZER APPLICATIONS ON GRASS DEVELOPMENT¹

CARTER M. HARRISON

(WITH THREE FIGURES)

Introduction

The production and maintenance of turf on golf courses must take into consideration the varied uses to which the several parts of such a course are put. The ground cover of the "putting greens," "fairways," and "rough" differs markedly. The turf of the putting greens should be of such a density, height, and quality that a golf ball will roll evenly and smoothly over its surface. These characteristics, furthermore, must be maintained throughout the entire playing season even though the blades may be clipped daily to approximately three eighths or even three sixteenths of an inch in length. The fairway turf should consist of grasses whose blades stand firmly and stiffly upright so that they will hold a golf ball up on their ends, "teeing" it up for the next shot. The fairway is usually bordered by the rough. As the name implies, the grasses which compose it are permitted to grow longer; often those of a bushy or tufted nature are included.

Much difference of opinion prevails as to the best treatment of turf to secure a desired result. Factors in the environment coupled with the treatment to which the plants are subjected not only determine the kind or kinds of plants that can be grown, but also influences the growth produced by them during any given season.

Precise methods for the production and maintenance of desirable turf on the fairways of the modern golf course have not been developed. Short cutting of the grass on the fairways during the playing season is generally practiced. At times, it is cut to approximately one half inch. The closer the grass is clipped, the longer will be the intervals between the time of cutting and the time when the grass is long enough to prove troublesome to the game. Lengthening the intervals between cuttings results in a material saving in operative costs. In most instances some practice of fertilizing in addition to the cutting of the grass is necessary. These practices may in extreme instances be quite as harmful as beneficial to the growth of the grass. In fact it is easily possible to weaken or even kill grass by certain treatments, so that the ground is either left devoid of plant life or becomes covered with undesirable plants.

¹ Contribution from the Hull Botanical Laboratory, the University of Chicago, under a fellowship granted by the United States Golf Association Greens Section.

Much difficulty is encountered from the prevalence of low growing weeds, clover, and coarse summer grasses under close cutting treatments given the fairways, whereas the rough which is generally left uncut for longer periods of time or cut higher, is relatively free from such troubles. Also the rough usually stays green and thick after the fairways have become dry and sparse.

It would seem probable, therefore, that if the fairways could be cut in such a manner that a greater amount of leaf surface could be left than is true with the usual close cutting treatment, they too would remain in better condition. This result might be accomplished either by cutting the fairways close and less frequently, or by cutting frequently with the mower raised. The first method might prove troublesome because of the length which the grass would attain during intervals between cuttings, whereas the second would be feasible since it would provide a suitable playing surface and yet prevent serious injury to the grass because of too close clipping.

Some of the obvious detailed problems connected with the maintenance of grass turf are, (1) What response, relative to the quantity and quality of new growth, will grass plants exhibit after being cut at different heights? (2) Do the different species and varieties of grass respond similarly to any given treatment with respect to both the top and root growth produced? (3) What effect will cutting have upon the amount and length of root systems produced? (4) How do the treatments affect the ability of the plants to extend themselves vegetatively? (5) What are the effects of various fertilizer treatments on the amount of top growth produced by the plant in relation to the amount of such growth removed or allowed to remain?

In this investigation, solutions to certain of these problems were sought. The particular studies and experiments undertaken had to do with (a) the effect of frequent cutting to three different heights, one-half inch, one and one-half inches, and three inches upon the development and the dry weight of the roots produced; (b) the effect of cutting combined with fertilizing upon the length of roots produced; (c) the external structural responses of the grass to cutting and fertilizing as they relate to the quantity and quality of the turf; and (d) any anatomical variations which might be brought about by cutting, or fertilizing, or a combination of both.

Although considerable work has been done on agronomic herbaceous plants with respect to frequency and time of cutting, and with respect to the effect of fertilizer applications on the yield and permanence of stand, there are virtually no detailed records of experiments dealing with fairway maintenance.

As early as 1879 LAWES and GILBERT (15) found that various fertilizers greatly affected the relative numbers of different species of herbaceous plants present in a permanent meadow. Nitrogenous fertilizers, in general, favored the growth of grasses and reduced all other plants to a minimum, while the absence of nitrogenous fertilizers enhanced the growth of leguminous and other miscellaneous species. They also found that when nitrogen alone was used, the yield was increased considerably the first ten years of the experiment but dropped almost to the level of the checks the second ten years. KOSINSKI (12) and GODLEWSKI (6) showed that the addition of nitrogen salts to plants restricted the growth of roots in length and favored the growth of the stems. GODLEWSKI further showed that presence of sugar in a nitrogen free solution favored the growth of roots. HARRIS (11) found that wheat grown to maturity without fertilizer had a greater proportion of roots to tops, but where a nitrogenous fertilizer was added top growth was greater in proportion to the roots though the total root growth was greater in the latter case. GREEN (9) noted that in general frequent cuttings induced quick growth. KRAUS (13), ALBERT (1), and McKEE (16) found that clipping of the tops of herbaceous plants retarded root development. SALMON *et al.* (23) found that cutting alfalfa at immature stages thinned the stand and lowered the yield. GREGORY (10) and CRIST and STOUT (3) stated that there appears to be a reciprocal effect between tops and roots of plants. WATERS (29), NELSON (17), and ALDOUS (2) showed that cutting frequently or cutting at immaturity depleted the reserves in the roots of herbaceous plants. NELSON also found a slow recovery after cutting, lower yields, and increased weed infestation, besides an increase on the plants of crown buds, shoots, and main stems, as a result of early and frequent cutting.

GRABER *et al.* (7), STURKIE (28), and PIERRE and BERTRAM (19) showed that the underground parts of alfalfa, Johnson grass, and kudzu could be reduced in weight below what they were at the beginning of the experiment by frequent and close clippings, and that the amount of reduction was associated with the severity of the cutting. PIERRE and BERTRAM found with kudzu that the roots of plants cut six times per season decreased in weight during a period of two years, those from plants receiving four cuttings increased about 150 per cent., those receiving two cuttings, about 400 per cent., and those receiving one cutting, about 1250 per cent. The percentage of reserve starch and nitrogen was found to be less than one-half as much in the roots of plants receiving six cuttings as in those of plants receiving four or fewer cuttings. The percentage of total sugars, however, was found to be greater. This was taken as an indication that a change from starch to sugar was taking place in the roots of plants receiving six cuttings, in order to produce new top growth. FITTS (4), working

with the fine turf grasses, found that the length of roots increased in relative proportion to the height to which the tops had grown. When the grass was surface fertilized, in order to produce a good turf, the roots were short, but when the grass was starved, the roots were longer and the turf was poorer. FAGAN, MILTON and PROVAN (5), in treating a mixture of grasses, grown from seed, with nitrate of soda, noted in the first harvest year that the response to the fertilizer was greater in the case of the grasses than of the clovers. This, coupled with the late growth of the clover, resulted in the latter, under most conditions of the investigation, being shaded and smothered by the grass. When the grass was cut at weekly intervals, the weight of clover in the total growth produced was equal to the weight of Italian rye grass (the most prominent grass). The yield of dry matter from the same area was much greater under a monthly system of cutting than under a weekly one. STAPLEDON and BEDDOWS (25), using orchard grass, concluded that repeated cuttings during the current season produced considerably less than the amount of a hay and aftermath crop. Repeated cutting reduced the root systems of the plants and retarded the growth produced early in the following spring. The pasture propagants gave as much as a 30 per cent. decrease in the amount of roots produced when compared with the hay plants and in some cases the difference amounted to 100 per cent. There was a wide response range to continued cutting in the pedigreed strains. Some resisted the treatment in a manner quite superior to that of others and showed only a slight reduction in roots. The plants with the highest yield did not necessarily have the heaviest root systems, but the nine highest yielding had roots somewhat heavier than the nine lowest yielding. Super-phosphate was without material quantitative or qualitative effects on root development, or on the stem to leaf ratio in the hay, aftermath, or pasture. Neither super-phosphate nor sodium nitrate alone was able to counteract the depressing influence of continued cutting.

REAM (20), STAPLEDON (24), STAPLEDON and MILTON (27) and STAPLEDON and DAVIES (26) found that root, tiller, or rhizome development and yield of the grasses studied were associated with the cutting treatment. The more drastic the cutting treatment, as measured by amount and frequency of defoliation, the less was the yield of roots, rhizomes and tops. The addition of nitrogenous fertilizers augmented the amount of yield of plant parts with a few exceptions where the cuttings were very short and frequent.

During the last fifteen or more years, several investigators have shown that chemical studies were pertinent to growth relations. KRAUS and KRAYBILL (14), NIGHTINGALE (18), and REID (21) have pointed out some apparent relationships between growth responses of plants and plant parts,

and the carbohydrate reserves and nitrogenous compounds within them. WELTON *et al.* (30) showed that the mowing of Canada thistles delayed organic food storage, resulting in a killing of the weaker plants. The more frequently the thistles were mowed, the more effective was the eradication. REID (22) showed that the relative amounts of carbohydrate to nitrogen compounds stored in seeds affected the ratio of tops to roots in the seedlings and that added nitrogen did little to augment growth unless light conditions were favorable for carbohydrate synthesis. GRABER (8) stated that frequent and close removals of the top growth of grasses having abundant reserves made for a heavy draft on the supplies of available nitrogen, so that the first limiting factor in growth might be nitrogen deficiency, but when regeneration was constantly stimulated by abundant mineral fertilizers, especially nitrogenous ones, the carbohydrate reserves were rapidly used with slight opportunity for replenishment and, as a result, they often became the principal factors limiting growth.

Experimental data

EXPERIMENT I

MATERIALS AND METHODS.—Preliminary greenhouse tests were made on three grasses in the spring of 1929. Three common golf course grasses were selected for the work, namely, Kentucky bluegrass (*Poa pratensis*), red fescue (*Festuca rubra*), and Colonial bent (*Agrostis capillaris*). The flats in which the grasses were grown were twenty-two inches long, fifteen inches wide, and two and one-half inches deep. They were filled with a good grade of black top soil. The seed was sown at the following rates: Kentucky bluegrass, 1.91 grams per flat; Colonial bent, 1.43 grams per flat; and the red fescue, 2.86 grams. These rates correspond to 2.0 pounds, 1.5 pounds, and 3.0 pounds per thousand square feet respectively.

The seed was sown on March 13th, the experiment was set up in triplicate, and the cutting treatment was started April 15th. The grass was cut to three heights, one-fourth inch, one and one-half inches, and three inches. In addition three flats of each grass were clipped short and ammonium sulphate was added on April 30th and May 22nd. The first application was made at the rate of two pounds per thousand square feet and the second at the rate of seven pounds per thousand square feet. The grass in all of the flats was cut sixteen times between April 15th and July 5th.

Beginning July 5th, the grass roots were washed from the soil with as much care as possible. The roots were distinctly matted in the grass cut to the higher levels, but this was not true of the most closely clipped flats.

RESULTS.—The results are recorded in table I in grams per square foot of surface area.

TABLE I
WEIGHT OF ROOTS IN GRAMS PER SQUARE FOOT OF SURFACE AREA

VARIETY	RED FESCUE		BLUEGRASS		COLONIAL BENT	
TREATMENT	GREEN WEIGHT	DRY WEIGHT	GREEN WEIGHT	DRY WEIGHT	GREEN WEIGHT	DRY WEIGHT
	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>
Short cut, $\frac{1}{2}$ inch	6.8	1.4	8.5	1.6	13.8	2.1
Short cut, $\frac{1}{2}$ inch and ammonium sulphate	4.3	1.0	6.8	1.3	Killed	
Medium cut, 1.5 inches	51.6	8.6	36.3	7.5	29.4	5.0
Long cut, 3 inches	78.0	13.7	61.0	11.7	59.1	7.7

Table I indicates that there is a considerable difference in the amount of roots produced as a result of the different cutting treatments and also by the different grasses. The root systems of the bent grass which was cut short weighed nearly double that of either of the other two grasses receiving the same treatment. The percentage increase in roots between plants when cut at three different heights is large and indicates a direct correlation between the amount of top growth and the amount of root growth produced.

The character of the response of the short clipped bent grass that was fertilized to the combination of a heavy nitrogenous fertilizer and shading of the greenhouse was interesting. The grass had been growing in a greenhouse that was not shaded. Until the greenhouse was covered to cut down the light, these flats were a beautiful green, while the flats having no nitrogen, and clipped short, were yellowish in color. No later than three days after the shade mixture had been applied to the greenhouse roof, the heavily fertilized grass rapidly took on a scalded appearance until only the plants near the sides of the flat showed a healthy green color. In the meantime, the grass that was clipped short and that had received no nitrogen turned from a yellowish color to a dark green. The plants which appeared scalded died and after examination of the material their death could not be attributed to fungus troubles. Appearing as it did about a week after a heavy fertilization with nitrogen and a decrease in the light intensity, it was thought that possibly in the absence of sufficient manufactured carbohydrates, the nitrogen was toxic and brought about the scalded condition.

EXPERIMENT II

GREENHOUSE EXPERIMENT WITH KENTUCKY BLUEGRASS AND RED FESCUE.—
 Because the flats used in the first experiment were too shallow to allow the

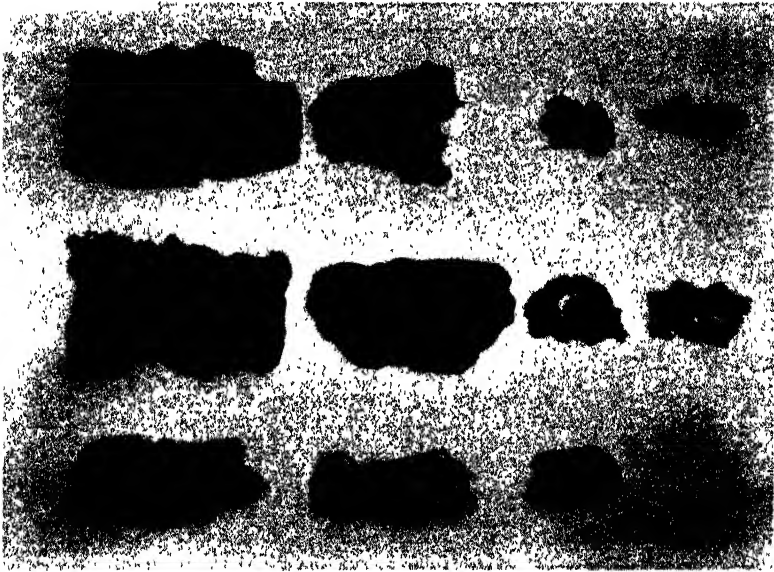


FIG. 1. Photograph showing bulk of roots produced by different grasses when cut to three heights and when nitrogen is added to short cut grass.

Upper row: Kentucky bluegrass. Reading left to right: cut long, cut medium, cut short, and cut short plus nitrogen.

Middle row: Red fescue. Treated same as bluegrass.

Lower row: Colonial bent. Treated same as bluegrass. Roots from the grass plants that were fertilized and clipped short are missing because the grass killed.

roots of the plants to grow downward and because they dried out too rapidly, another experiment was begun in the fall of 1929 to duplicate in part and supplement in some features the preliminary study.

MATERIALS AND METHODS.—Wooden boxes of approximately one cubic foot volume were obtained. These were filled with a mixture of eight parts of black soil, two parts of quartz sand, and one part of leaf mold. The soil was sifted to remove large particles of organic material and gravel. The seed was sown on September 25th.

Fertilizer applications were made once per month during January, February, and March. The applications consisted of the following:

1. Nitrogen, 1.12 grams of ammonium sulphate.
2. Nitrogen-phosphorus, 1.12 grams of ammonium phosphate.
3. Nitrogen-potassium, 0.85 grams of potassium nitrate plus 0.57 grams of ammonium sulphate.
4. Nitrogen-phosphorus-potassium, 1.12 grams of ammonium sulphate plus 1.15 grams of monobasic potassium phosphate. The amount of nitro-

TABLE II

DRY WEIGHT OF ROOTS IN GRAMS PER SQUARE FOOT FOR THE DIFFERENT CUTTINGS AND FERTILIZER TREATMENTS IN KENTUCKY BLUEGRASS AND RED FESCUE

	BLUEGRASS						FESCUE					
	Cut 1/4 INCH	± MEAN	Cut 1 1/2 INCH	± MEAN	Cut 3 INCH	± MEAN	Cut 1/2 INCH	± MEAN	Cut 1 1/2 INCH	± MEAN	Cut 3 INCH	± MEAN
Check	1 2.6	-0.03	7.8	-0.07	10.7	-1.47	4.7	-0.23	7.5	-0.90	13.8	-0.63
	2 3.0	+0.37	8.3	+0.43	11.5	-0.67	4.5	-0.43	9.1	+0.70	16.1	+1.67
	3 2.3	-0.33	7.5	-0.37	14.3	+2.13	5.6	+0.67	8.6	+0.20	13.4	-1.03
Nitrogen alone	1 4.3	+0.47	6.8	-0.60	10.0	+0.57	7.0	+0.93	9.2	+0.43	11.0	-0.03
Ammonium sulphate	2 3.3	-0.53	7.4	± 0.0	9.5	+0.07	5.2	-0.87	8.9	+0.13	10.6	-0.43
	3 3.9	+0.07	8.0	+0.60	8.8	-0.63	6.0	-0.07	8.2	-0.57	11.5	+0.47
Nitrogen and phos- phorus	1 3.2	+0.23	6.5	-0.50	8.9	-0.63	5.2	+0.23	8.5	+1.0	10.3	+0.60
Ammonium phosphate	2 2.4	-0.57	7.1	+0.10	10.6	+1.07	4.8	-0.17	6.8	-0.70	9.5	-0.20
	3 3.3	+0.33	7.4	+0.40	9.1	-0.43	4.9	-0.07	7.2	-0.30	9.3	-0.40
Nitrogen and potas- sium	1 4.0	+0.30	8.1	+0.17	8.5	+0.50	5.6	+0.20	7.0	+0.03	9.8	+0.03
Potassium nitrate and ammonium sulphate	2 3.9	+0.20	8.6	+0.67	8.3	+0.30	5.4	± 0.0	8.1	+1.07	10.5	+0.73
	3 3.2	-0.50	7.1	-0.83	7.2	-0.80	5.2	-0.20	6.0	-1.03	9.0	-0.77
Nitrogen phosphorus and potassium	1 3.1	+0.67	7.8	-0.03	10.0	+0.70	5.6	+0.07	7.1	+0.23	11.5	+0.43
Potassium acid phosphate and am- monium sulphate	2 2.2	-0.23	8.4	+0.57	9.2	-0.10	5.9	+0.37	6.3	-0.57	10.5	-0.57
	3 2.0 ¹	-0.43	7.3 ¹	-0.53	8.7 ¹	-0.60	5.1 ¹	-0.43	7.2 ¹	+0.33	11.2 ¹	+0.13

¹ Weight of roots of the flats from which the photographs were obtained.

gen added to the grass in each flat was the same regardless of the salt used. The experiment was set up in triplicate.

Beginning November 7th the plants were cut and the cutting continued each week thereafter until the close of the experiment. Fifteen flats of each species of grass were cut to one-half inch, fifteen to one and one-half inches, and fifteen to three inches. All clippings were removed from the flats. Beginning April 21st the soil was washed from the roots. The dry weight of the roots was recorded, the results being given in table II.

From table II it is obvious that the cutting heights made considerable difference in the amount of roots produced and that very little can be said in favor of the fertilizer treatments with respect to root growth. More difference is apparent between the triplicates of a certain treatment than between treatments.

Besides weighing the roots produced by the different treatments, it was thought advisable to select a flat of grass of each of the three cutting heights where the best top growth was obtained from which to get photographs of the height of cut, the density of the turf, and the length and amount of roots produced. The complete fertilizer gave what appeared to be the best top growth at all three heights. The fescue was selected likewise. The rhizomes were carefully picked out of the three fertilized bluegrass flats, and likewise out of three check flats, and the oven dry weight was recorded as in table III.

TABLE III

TREATMENTS	TOTAL DRY WEIGHT OF RHIZOMES PER FLAT
Bluegrass—clipped long, potassium phosphate and ammonium sulphate	gm. 0.726
Bluegrass—clipped long, check	0.594
Bluegrass—clipped medium, potassium phosphate and ammonium sulphate	0.400
Bluegrass—clipped medium, check	0.225
Bluegrass—clipped short, potassium phosphate and ammonium sulphate	0.018
Bluegrass—clipped short, check	0.008

Many more rhizomes were produced by the grass cut high than by that cut to a medium height, and decidedly more were produced by the grass cut to a medium height than by that cut short. In fact, very few of the plants in the short cut flats had visible rhizomes.

Sections were cut of representative plants selected from the checks and from those receiving nitrogen, in the three cutting heights. This was done on both the fescue and the bluegrass. Nothing could be noted from the

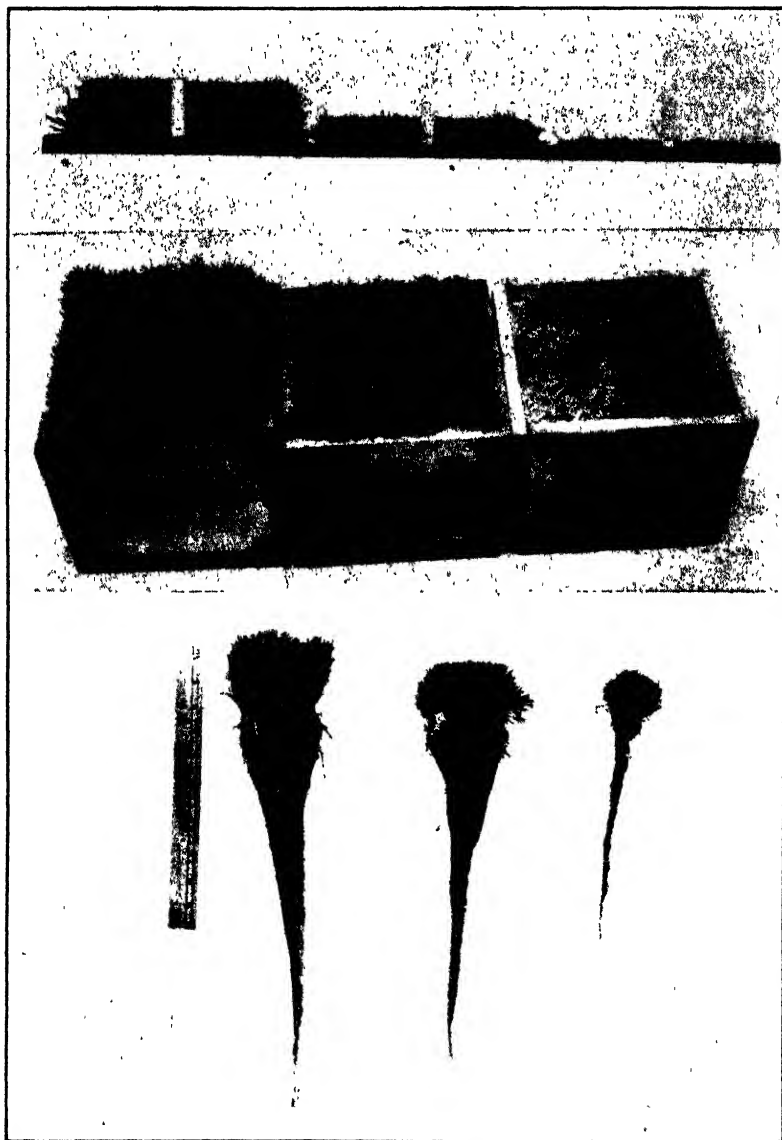


FIG. 2. Three bluegrass flats to which a complete fertilizer had been added, showing: above, the height of cut; middle, the turf produced at those heights; and below, the mass and length of roots produced.

slides that was not apparent in the external structure of the plants. It was observed that the shorter cutting of the fescue induced much more tillering than was the case in those cut longer. The grass cut to a medium

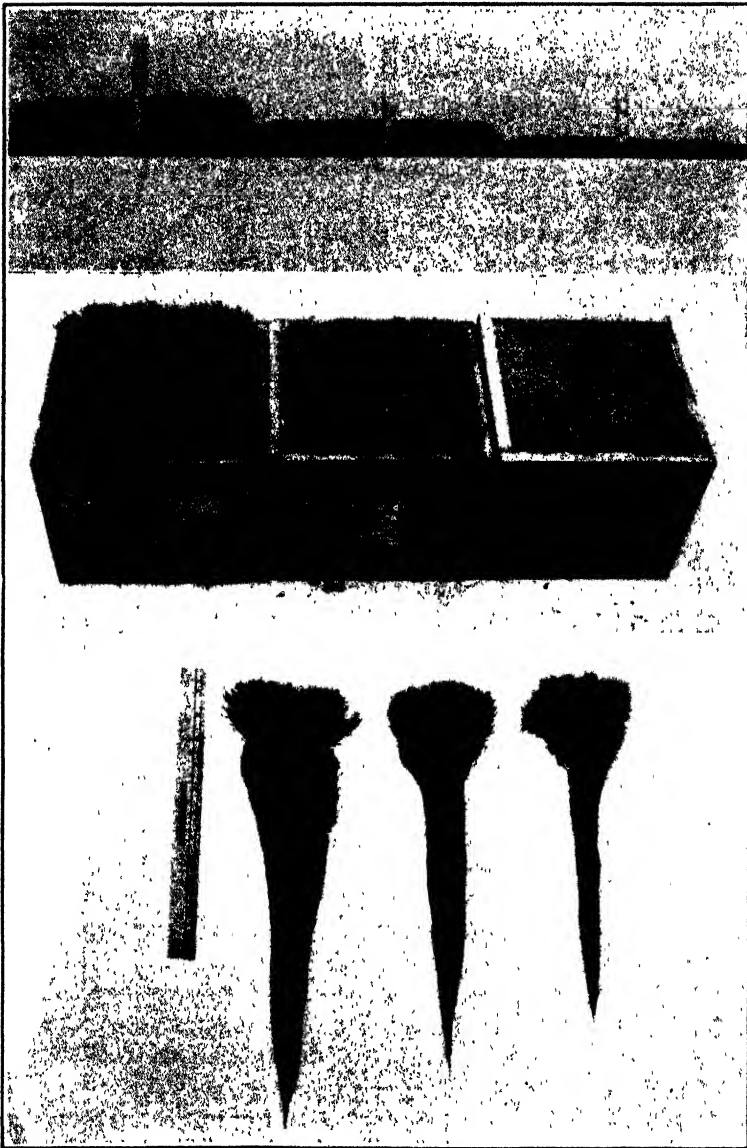


FIG. 3. Three fescue flats to which a complete fertilizer had been added, showing: above, the height of cut; middle, the turf produced at those heights; and below, the mass and length of roots produced.

height had stiffer and more upright leaf blades than that cut long, the long cut plants having a tendency to lop over. This was probably due to length

of blade rather than to a difference in the anatomical structures of the two. The plants clipped short tillered more than those cut to a medium height, but the leaf blades were not nearly so stiff. The grass cut to a medium height would hold up a golf ball, while neither of the others would do so. None of the cutting treatments appeared to result in a thinning of the fescue turf. The grass in the check flats could be picked out late in February after a period of bright weather, and there was also a difference in length of top growth in favor of the fertilized grass. The bluegrass, on the other hand, killed considerably when clipped short, that with the medium cutting height produced a good turf with stiff leaf blades, and that cut long produced a turf that lopped over as did the fescue which was clipped high. The effect of the nitrogen on the bluegrass was not apparent until late in February after the second fertilizer application. All the grass had been a dark green color until this time when the weather became bright and clear as contrasted with the cloudy, sunless period preceding it. During these sunny days, the checks in all three cutting heights turned a greenish yellow while the grass in the flats receiving nitrogen remained a dark green. Between cutting periods, the grass clipped long receiving nitrogen grew approximately three inches, while that in the checks grew one inch. The medium cut grass showed the same response but to a less degree, the growth in height being about two inches in the fertilized flats of grass as compared to three quarters of an inch in the checks. In the flats of grass cut short and fertilized, the plants sent up one spindly blade, the turf thinned out badly, and it appeared as if the nitrogen was harmful here because the grass in the check flats did not thin out or have this spindling habit of growth.

It was noted that the shorter the clipping treatment the smaller the subsequent plant parts became. The roots were finer and shorter and the leaf blades were narrower and shorter. The growth in height corresponded to the size of the plant. The plants in the check flats, regardless of the cutting height, appeared to be storing carbohydrates as evidenced by a yellowish green color of their leaves when compared with the darker green color of the leaves of the plants receiving nitrogen. This might possibly indicate that as the severity of the cutting treatment increased, the new roots produced by the plant became fewer, shorter and finer. Thus a gradual adjustment between the amount of top growth and root growth took place, the final result being that in no case was the root system sufficiently extensive to gather enough nitrogen to prevent carbohydrate storage.

Summing up the general observations, it appears as if the nitrogen had resulted in increased top growth in all cases except the short clipped bluegrass. The fescue which was cut short tillered more than that cut long. That cut at a medium height, however, had the most desirable leaf blades from a golf course view-point.

The foregoing data show that the shorter the grass was clipped, the fewer roots were produced. It has been shown by several workers that frequent removal of top growth influenced the subsequent yield of tops, lessened the carbohydrate storage, and if the clipping were severe enough, no carbohydrates were stored. It has also been noted that when the tops are severely clipped, the weight of the root systems may be reduced below the initial weight at the start of such treatment. Lessening the leaf area resulted in the production of fewer, smaller roots than was the case when the treatment was not so severe. The size of the successive leaves produced by the plant depended on the cutting treatment: the more severe the treatment, the smaller they became. As the clipping treatment became more and more severe, generally less and less leaf area was left to manufacture carbohydrates, which are necessary both for the growth of roots and for subsequent top growth.

Removal of photosynthetic surface can be carried to such an extreme that the grass plant is weakened to such a degree that it can no longer survive either drouth, disease, extremes of heat or cold, or the competition of plants which have growth characteristics enabling them to escape the close clipping by tillering, or by growing almost flat on the surface of the ground. These plants, such as white clover, dandelion, crab grass, knot-weed, and plantain, retain sufficient leaf area to maintain themselves under clipping practices which are very detrimental to the growth of upright growing grasses such as Kentucky bluegrass. Bluegrass continues to grow erect, and the new chlorophyll bearing tissue is continually cut off as it is produced. Field observations as well as greenhouse studies show that Kentucky bluegrass cannot maintain itself under ordinary field conditions if it is cut shorter than three-quarters of an inch more frequently than once each week. The plants extend themselves very little vegetatively when these practices are followed. The rhizomes formed are few in number and grow very slowly. If the grass is allowed to grow longer or is cut less frequently, there is sufficient production of rhizomes to insure good perennial turf, and besides, the undesirable plants are kept hidden or are crowded out by the vigorous growth of the grass. Even among the different grasses, there are species that can maintain themselves where others are killed by the short and frequent clipping. Most of the vegetative and seeded bent grasses that are in use today for turf purposes produce considerable chlorophyll-bearing tissue near the surface of the ground in such a manner as to escape close clipping. It is this characteristic that makes them valuable for the putting greens. Red fescue tillers when cut short, and produces many leaves close to the ground away from the mower blades. Therefore it is able to withstand more severe cutting than grasses which do not have this habit of growth.

The relationship that exists between carbohydrates and nitrogen in plant nutrition has been pointed out by workers in the field, and it has been shown that an application of nitrogen is of little use in subsequent growth unless the plant has an available supply of carbohydrates or is capable of manufacturing it. An excess of mineral fertilizer cannot take the place of carbohydrates in plant growth. The appended data show in general that root growth will not respond to fertilizer applications if the ability of the plant to manufacture carbohydrates is impaired by short clipping. The mowers should be raised on the fairways where the cutting treatment is proving dangerous, and cutting should be discontinued in the fall as early as possible in order that the plants may store foods for use during the season of short and frequent clipping.

Summary

1. Clipping at different heights affected the amount of roots produced on the grasses studied. The shorter the grass was cut and the more the leaf area was reduced, the smaller was the quantity of roots produced.
2. Grasses having different growth characteristics responded differently to the cutting treatments. The fescue tillered more when cut short than when cut long, and the bluegrass produced fewer rhizomes when cut short than when cut long.
3. Mineral fertilizers did not compensate for a lack of top growth in the production of roots.
4. The killing of the grass was not due to a cutting off of the buds, but to a gradual carbohydrate starvation to a point beyond which the plants could not maintain themselves.
5. The addition of nitrogen brought about an increase in top growth, but the weight of roots was not increased over that of the roots of the unfertilized grass.

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EFFECT OF X-IRRADIATION UPON GROWTH AND REPRODUCTION OF TOMATO*

EDNA LOUISE JOHNSON

(WITH THREE FIGURES)

Experimentation (2) has shown that plants differ in the way that they react to X-ray exposure. The tomato, *Lycopersicum esculentum*, is less affected by the action of these rays than are some other species. Since in a preliminary study seeds given a medium dose produced plants which appeared normal in all respects, all later irradiation was made directly on the seedling.

Materials and methods

Seven different sets of experiments (series I-VII) were carried on, in most of which the John Baer variety of tomato was used. In the first three series irradiation was carried out when the plants were from 1 to 3 centimeters in height; in the fourth, three medium doses were given at two-week intervals,—at a time when the plants seemed to be recovering from the effects of the previous dose. In the last three groups, the plants were not exposed until small buds had formed.

The general procedure was to plant seeds in 5-inch pots in the greenhouse. The plants of the first three series were irradiated before the true foliage leaves had developed. Soon afterwards these seedlings and an equal number of controls were transplanted to larger pots where they grew to maturity. Plants of series V, VI, and VII were transplanted at an early age but were not irradiated until small buds had formed.

Irradiations of the first five groups were made with a Victor X-ray machine with the following "set-up": 5-inch spark gap, 5 milliamperes current, no filter, distance 30 cm., time from 20 to 30 minutes. For the last group, an Acme machine was used with the potential 90 K.V., 5 milliamperes current, no filter, distance 30 cm., time 20 minutes.

Results

EFFECT ON THE LEAVES OF NEW GROWTH

The first noticeable effect on the plant is a peculiar warty or pebbly appearance of the young foliage leaves which is evident soon after irradiation. As the leaves grow older, they show light green areas intermingled with the ordinary green thus giving a mosaic or variegated aspect. Microscopic

* The latter part of this study was made possible by means of a grant from the "Committee on the Effects of Radiation upon Living Organisms" of the National Research Council. Grateful acknowledgement is made to the Physics Department of the University of Colorado and to the Boulder X-ray Association for radiation of plants.

examination of the leaves indicated that there had been an interference with the normal development of palisade and spongy parenchyma. The walls of the palisade cells particularly seemed to lack distinctness of outline. The chloroplastids appeared contracted and more blue-green in color than did the plastids of the controls.

The cotyledons remained entirely normal, but marked leaf abnormalities were evident in young tissue developing after irradiation. In many cases, for half the length of the leaflet, the blade on one side was absent. Leaflets were often twisted for one-half or one-third of their length. Fusion of leaf parts was common. Often the widened base of the leaflet grew to the main rachis so that no petiole at all was present. One case was noted where a lateral leaflet showed the tip edges attached together to form a cup-like structure. The edges of the leaflets were sometimes grown to the main rachis or with adjacent leaflets. In some cases marked leaf anomalies were not evident, but one could always distinguish irradiated plants by the pebbly appearance of the young leaves.

CHANGES IN GROWTH FORM

A change in the general aspect of the entire plant, resulting from greater development of lateral branches is another condition noticeable in every group of tomato plants given medium dosage. A special study of this trait was made in series IV, V, and VI. In series IV, the dose was repeated twice at approximately two-week intervals. The last dosage was given when the buds had formed. Earlier experiments (2) had established the fact that immediately after irradiation with a dose of medium strength, a growth depression occurs which later tends to disappear. Those employing X-rays for therapeutic purposes find that a similar condition holds true for a treatment of human diseases. The dose is repeated between the second and third week, as the effects of the treatment usually disappear by the end of the third week. It seemed desirable to compare effects produced by giving doses to plants at various intervals with the results produced by a single exposure at the budded stage. Series IV was given three medium doses at intervals while the plants in series V, VI, and VII received but one each at the time small buds had formed.

The bushy appearance presented by the irradiated plants is due to the greater development of lateral branches. A comparison of this feature in the irradiated and control plants is shown in table I. All lateral branches one centimeter or more in length were counted and measured. In series IV, there was an increase in branch development of 27.5 per cent. over that found in the control plants. In series V and VI where but one exposure was given to plants which had already formed buds, the percentage of increase of total length of branches from irradiated plants over the control

TABLE I
LATERAL BRANCH DEVELOPMENT

	SERIES IV			SERIES V			SERIES VI		
	CONTROL	IRRADIATED ¹	INCREASE OF IRRADIATED OVER CONTROL	CONTROL	IRRADIATED ²	INCREASE OF IRRADIATED OVER CONTROL	CONTROL	IRRADIATED ²	INCREASE OF IRRADIATED OVER CONTROL
Days after radiation when measurements were made			<i>per cent.</i>			<i>per cent.</i>			<i>per cent.</i>
Age when radiated		47.0			35.0			50.0	
No. of plants	11.0	17.0		12.0	62.0		11.0	55.0	
Total no. of side branches	76.0	11.0	2.6	10.0	12.0	120.0	20.0	28.0	40.0
Av. no. per plant	6.9	78.0	2.8	0.75	22.0	140.0	1.81	2.54	40.0
Total length (cm.)	410.0	7.1	27.5	100.0	1.83	56.4	66.0	109.0	65.1
Average length (cm.)	5.39	522.8	24.3	10.0	156.4	-29.0	3.35	3.9	16.4
		6.70			7.1				

¹ Irradiated with 3 medium doses (5 in. spark, 5 milliamper., 30 cm. for 20 min.) at intervals of approximately two weeks.

² Irradiated with one dose (5 in. spark, 5 milliamper., 30 cm. for 20 min.)

³ Irradiated with one dose (90 K.V., 5 milliamper., 30 cm. for 20 min.)

was considerably greater than in Series IV, whereas the average length was less.

Results in table I indicate that tomato plants irradiated either in the seedling stage or at the time that buds are appearing produce a greater number of lateral branches per plant than controls of the same age. In two of the three groups, the average length of branches from the irradiated plants was also greater.

EFFECT ON FLORAL DEVELOPMENT

Observations were made on four series of plants to determine the effects of irradiation on the time of blossoming and the occurrence of anomalies in buds and blossoms. The normal tomato flower is perfect, regular, and typically six-merous. The calyx tube is very short, and bears six leafy lobes which are linear to lanceolate. The six or more yellow stamens are laterally joined to form a hollow cone around the pistil. The pistil has an elongated style, and the stigma extends slightly beyond the apex of the androecium. Many types of anomalies were found in blossoms developed from buds which had been X-rayed. Table II gives a list of abnormalities present in blossoms of series IV, seventeen days after the last exposure. In this group, three exposures were made, the first on 17-day old seedlings, the last when buds were barely visible. Only those blossoms which were fully open at the time of observation, or those which had just faded, were included in the list.

TABLE II
ANOMALIES FOUND IN BLOSSOMS OF IRRADIATED PLANTS OF SERIES IV*

DESCRIPTION OF ANOMALY	NUMBER OF CASES
Double blossom	2
Corolla showing more than 6 lobes	16
Lobes of corolla cleft	3
Corolla lobes badly twisted	3
Calyx with more than 6 lobes	4
Lobes of calyx cleft	2
Fusion of calyx lobes	13
Twisted stamens	12
Total	55

* At the time of observation, there were 24 blossoms on the 11 irradiated plants under observation.

On the same day that the above observations were made on the irradiated plants, there were 16 blossoms present on the 11 control plants. Fifteen of these had six corolla lobes and six calyx lobes.

In view of the results given above, it seemed probable that similar anomalies could be produced by allowing plants to come to the budding stage and then irradiating them with one medium dose. With the last three series, plants were irradiated when they were approximately 60 days old. With some plants, the only part of the bud which showed any growth after irradiation was the pistil. Frequently the style became greatly elongated protruding from the calyx tube. Often the style showed marked flattening. In many cases, the buds present at the time of irradiation became yellow and dropped off. In one case, the irradiation affected the plant so that buds did not develop. Table III summarizes the floral development of the last three series.

TABLE III
DEVELOPMENT OF FLOWERS FROM FIRST BUD CLUSTER

	SERIES V. 62-DAY OLD PLANTS IRRADI- ATED WITH ONE DOSE FROM FOLLOWING SET-UP: 5 INCH SPARK GAP, 5 MILLIAMPS., 30 CM. FOR 20 MIN.		SERIES VI. 60-DAY OLD PLANTS IRRIDI- ATED WITH ONE DOSE FROM FOLLOWING SET-UP: 90 K.V., 5 MILLIAMPS., 30 CM. FOR 20 MIN.		SERIES VII. 55-DAY OLD PLANTS IRRADIATED WITH ONE DOSE FROM FOLLOWING SET-UP: 90 K.V., 5 MILLIAMPS., 30 CM. FOR 20 MIN.	
	CONTROL	IRRADIATED	CONTROL	IRRADIATED	CONTROL	IRRADIATED*
No. of plants ...	12	12	11	11	7	7
Total no. of blossoms	56	1	43	13	25	23
Average no. of blossoms	4.66	.083	3.9	1.18	3.57	3.28
Ave. no. of days for blossoms to appear	17.4	..	24.3	20	25.4	29.3
Percentage of plants never developing flowers in first bud-cluster	0	91.6	0	63.6	0	0

* Lessened effect probably due to fact that plants were irradiated when buds were younger than those in the other series. Leaf anomalies were as evident as in other irradiated plants.

A comparison of flower development in series IV and V indicates an existence of radiophylaxis which ANCEL and LALLEMAND (1) have demon-

strated. In their experiments, it was found that an exposure to a light dose preceding the heavy dose lessened the effect of the heavier dose which followed. In series IV one medium dose which was given to the 17-day old seedlings was repeated at two-week intervals, the last being given when small buds had formed. When these plants were 3.5 months old, 9 per cent. of the irradiated plants, and 63.6 per cent. of the controls, were bearing fruit. When these same plants were 5 months old, 54.5 per cent. of the irradiated, and 90.9 per cent. of the controls, were fruiting. With series V where the plants were exposed to but one medium dose, irradiated plants 3 months old had produced no fruit while 75 per cent. of the controls were bearing fruit. When these plants were 4 months old, 25 per cent. of the irradiated, and 92 per cent. of the controls, were in fruit.

The plants which had two previous exposures before irradiation did not drop their buds but developed flowers. These showed many malformations of structures, it is true, but the injury was considerably less than that sustained by the plants of series V in which only one bud that received irradiation ever developed a flower. The X-rays apparently hastened the development of the abscission layer of the pedicel for, soon after the exposure, the region of the pedicel where the abscission layer develops became yellow and the bud soon dropped. This case seems to illustrate the phenomenon of radiophylaxis, as the earlier doses given the plants apparently prevented the serious injury which results when one dose only is given at the time the buds are formed.

An accurate record of the time of blossoming and the number of blossoms appearing in the second bud cluster was kept for series VI and VII. In both of the series there was but little difference in the time of blossoming between the experimental plants and controls. The average number of blossoms, however, was greater in the irradiated plants. This will be noted particularly in future study in order to determine whether inhibition of flower and fruit development from the first bud cluster will be accompanied by increased vegetative and reproductive growth of the irradiated plants. In series IV and V double and triple flowers were produced. Figure 1 shows two of the unusual flowers produced. One was a double structure as is shown by the presence of two pistils with the surrounding androecia. The second flower was a triple one with three separate pistils each surrounded by a complete androecium.

FRUIT DEVELOPMENT

All groups of tomato plants were allowed to grow to maturity so that fruit development could be observed. In series I, buds appeared on irradiated plants at about the same time as on the controls. Fruit development seemed in no way affected by irradiation. In the plants of series II where

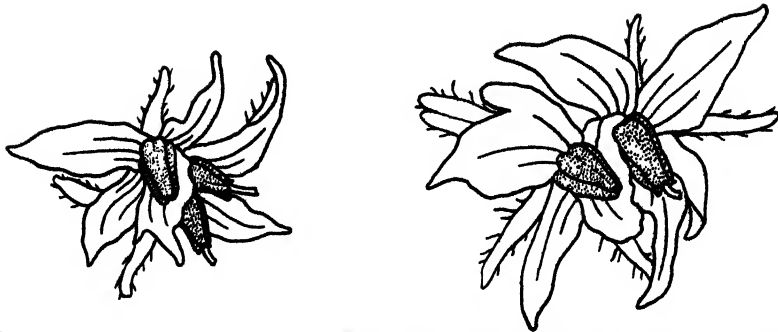


FIG. 1. Flowers produced on new growth which developed after irradiation of plants with a medium dose. At left, triple flower showing three pistils with their surrounding stamens. In two of the flowers, the stamens are twisted so that a portion of the style is exposed. At right, double flower with two pistils and their surrounding stamens.

a slightly heavier dose was given, fruits appeared on the irradiated plants at a somewhat later date than on the controls. When a still heavier dose was given to plants of series III, fruit development was still more retarded.

In plants of series IV where a medium dose was given three times, fruit production was slow for a time. Later, when the plants had time to recover from the last dose, the number of fruits more nearly equalled those



FIG. 2. Two-month-old tomato plants irradiated when the buds were very small presented this appearance 5 weeks later. Note the lessened height, branching habit, and sterile condition of the experimental plants. Buds have not developed on the deformed, abnormal growth. Controls show normal growth form and well developed fruit with new buds forming.

of the controls. In series V which was composed of a group of plants which were not irradiated until small buds were produced, none of the irradiated flowers produced fruits. In fact, only one of the buds produced a flower. That bud was well advanced in its development at time of the irradiation. Two months after irradiation some of the new growth which had developed, produced flowers and in 25 per cent. of the plants, fruits, often abnormal in shape, were produced. Five weeks after irradiation of plants in series V, there was a noticeable difference in height, branching habit, and fruiting condition of the control and X-rayed plants (figure 2). Buds which had been present at the time of irradiation had dropped without opening. No new buds developed until considerably later.

Data given in table IV show that irradiation of young tomato seedlings with a dose of medium intensity may delay fruit formation somewhat but in no way inhibit it on growth produced a few weeks after exposure. One medium dose given at a time when small buds are formed, causes plants to be almost completely sterile until new growth develops. A small percentage of the irradiated plants will, in time, produce fruits on the new abnormal growth.

TABLE IV
FRUIT DEVELOPMENT

SERIES NUMBER	AGE OF PLANT IRRADIATED	DOSAGE* MILLIAMPERE- MINUTES (DISTANCE 30 CM., NO FILTER)	TIME AFTER RADIATION WHEN REC- ORD WAS TAKEN	FRUITS PRODUCED	
				IRRADIATED	CONTROL
	<i>days</i>		<i>days</i>	Av. no. produced per plant	
I	17	7950	200	5.3	4.6
II	17	9000	...	Fruits delayed	Appeared in normal time
				Percentage of plants bearing fruits	
III	17	12000	90	80	90
IV	17	6000	99	9	63.6
			107	0	27.2
			136	54.4	90.9
V	62	6000	35	0	75
			64	25	91.7
VI	60	6000	45	18	100
VII	55	6000	50	71	100

* In series I-V, 5 in. spark gap used; in VI and VII, potential of 90 K.V.

When plants are given one medium dose at an early age, fruit development is but little affected; but if this first dose is increased, fruiting is

delayed although not inhibited. In one series of plants where one medium dose was given just as buds were forming, none of the irradiated buds produced fruits. New growth which developed during the next two months, produced abnormal flowers and fruits.

In other series, where one exposure was made after buds had formed, the effect seemed to vary with the age of the buds. If buds were of fair size, they became yellowed and dropped off; but when very small at the time of exposure they produced flowers showing some anomaly of structure. If fruits developed at a later period, they were misshapen and in many cases without seeds. Figure 3 shows a cross-section view of four fruits from irradiated plants of series VII with three controls.

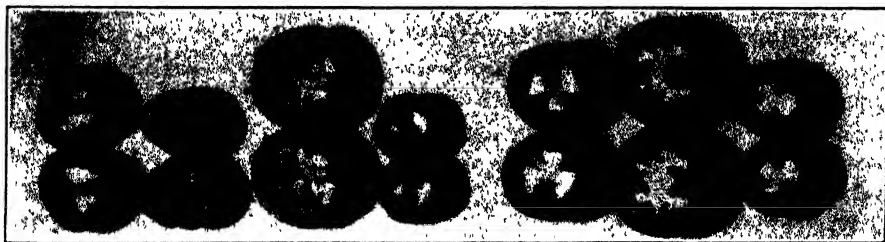


FIG. 3. Cross-sections of tomato fruits. At right, controls; at left, fruits which developed on plants which had received one medium X-ray dose after the formation of small buds. Note the absence of definite loculi, pockets in the pericarpal wall, disorganization of core and placenta, and the reduced number of seeds.

The control presents the appearance of the normal tomato fruit. The flesh consists of the outer pericarpel wall, the radial walls or interlocular septa, the placentae and the core. The number of loculi shows clearly. In the fruits from irradiated buds, however, one cannot distinguish definite loculi. The placenta and core show lack of normal structure and, in some fruits, there is an almost total absence of seeds. Pockets, which have formed in the outer pericarpel wall of the experimental fruits, are not present in the controls.

Summary

1. Irradiation of young tomato seedlings causes marked leaf anomalies in the young growth which develops after irradiation. Leaflets are often absent, badly twisted, or joined together in unusual fashion. Soon after irradiation, the leaves present a peculiar warty or pebbly appearance. As they grew older, there are areas of green of varying intensities so that they appear spotted or variegated.

2. Plants receiving X-ray dosage develop many lateral branches which cause them to assume a bushy appearance. The number of branches developed may be sixty-five per cent. greater in the irradiated plants than in the controls.

3. Many abnormalities of floral parts, including production of double blossoms, occur when plants are thrice irradiated previous to blossoming. If, however, radiation occurs at time of budding when there has been no previous dose, the buds become yellow and drop off without blossoming. Later growth may produce blossoms, some of which are normal while others are double or triple.

4. Fruit development is somewhat delayed in plants which are irradiated during their early seedling stages. However, in plants which were irradiated just before blossoming with one medium dose, complete sterility was present for some time. Later the new growth produced small abnormal fruits on twenty-five per cent. of the plants as compared with one hundred per cent. fruit production of the controls.

5. Radiophylaxis, a lessening of the effect of a harmful dose by giving a preceding lighter one, was demonstrated by tomato flowers in one series. A dose which would ordinarily cause a yellowing and dropping of buds if given as the buds were forming, did not have such a markedly fatal effect when two earlier doses were given before the reproductive stage.

6. Fruits which do develop on irradiated plants show a lack of definite internal pattern. The placenta and core show abnormal development and there is an almost total absence of seeds. Pockets formed in the pericarp of fruits from irradiated plants are not found in the controls.

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EFFECT OF FERTILIZERS AND DATE OF PLANTING ON THE PHYSIOLOGICAL DEVELOPMENT OF THE CORN PLANT¹

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(WITH TWELVE FIGURES)

Introduction

As a part of the Ohio program of corn borer research several years of investigations on the growth, rate of maturity, and yield of early and late planted corn have been carried on at Wooster. Studies have been made of the physiological conditions associated with the development of the plant, with differences in yield, and with variations in the length of time required for the maturing of late season varieties, that resulted from varying, (1) the date of planting; (2) the time and method of fertilizer application; and (3) the kind and quantity of fertilizer used.

Among the many contributions to our knowledge of the physiological and chemical phenomena of the growth of plants are numerous investigations leading to the establishment of the S-shaped curve of growth and attempting to offer an explanation of it. The papers on this subject are too numerous to review in detail.

• WOLFE (9) found that within a given variety, the highest yielding corn plants silk early, and that the corn plants which mature first are the ones that tassel, silk, and shed pollen earliest. He concluded that any one of these characters indicates the relative time at which plants will reach final maturity. McCLELLAND (3) obtained satisfactory yields when late planting of corn was followed by heavy rainfall, but if followed by a drought, the yield was greatly reduced. McCool (4) found that the sap of corn plants grown on a soil receiving fertilizer had a greater osmotic concentration than when grown on unfertilized soils. He concluded that this greater concentration in the sap would prevent late spring frost injury to seedling corn plants.

MILLAR (6) obtained a more vigorous growth in corn which received hill applications of fertilizer, than in corn which received fertilizer broadcast. Early maturity also followed as a result of hill fertilization. No limitation in root development was noted as a result of placing the fertilizer in the hill. His method of applying the fertilizer was to scatter it over an area of about 5 by 10 inches, in a shallow depression, and cover with a light layer of soil before dropping the seed.

¹ Contribution from the Department of Agronomy, Ohio Agricultural Experiment Station.

HARPER (2), who applied the fertilizer on an area of about 10 by 3 inches and 1.5 inches above the seed, found no deleterious effect from hill fertilization on the root systems of the corn plant. He suggests that very heavy applications of fertilizers high in nitrogen may produce smaller root systems.

TRUOG, *et al.* (8) found that germination of the seed was unimpaired when a complete fertilizer was applied at the rate of 120 to 200 pounds in the hill above the seed, and that maturity may be hastened as much as two weeks through hill applications of fertilizers on corn at planting time, providing some method is used to prevent direct contact of seed and fertilizer in the hill.

Plots

Burr-Leaming corn was grown in each of 3 years on a series of plots located on Canfield silt loam of medium to low fertility. The soil was fairly well drained and had an approximate reaction of pH 5.5. Two tons of ground limestone were applied as a basic treatment.

The series of plots comprised four separate sections, each containing twenty-eight 1/20 acre plots. Ten plots of each section were used as checks, the remaining eighteen being given different fertilizer treatments. Each year two sections with duplicate plot treatments were planted to corn; one section on or about May 15, the other on or about June 5.

The fertilizers² applied to the plots consisted of incomplete and complete types, such as 0-16-0, 0-12-4, and 3-12-4. Manure supplemented with 16 per cent. superphosphate was also applied to some plots. The quantity of chemical fertilizer applied varied from 100 pounds per acre, which may be considered as establishing a low level of fertility, up to 400 pounds per acre which may be considered as establishing a high level of fertility.

The fertilizers were applied to the plots at planting time either all broadcast, all in the hill, or part broadcast and part in the hill. The latter method is termed a "split application." Some of the plots received applications of 8 tons of manure and 225 pounds of 16 per cent. superphosphate broadcast. This treatment was repeated also on other plots and supplemented with varying amounts (100, 200 and 400 pounds per acre) of a 3-12-4 fertilizer applied in the hill. Hill applications were made with a modern corn planter through the fertilizer attachment which provided for some separation of seed and fertilizer.

² Fertilizer were mixed from nitrate of soda, 16 per cent. superphosphate and muriate of potash. A fertilizer of 3-12-4 analysis contained a quantity of nitrate of soda equivalent to 3 per cent. NH_4 , superphosphate equivalent to 12 per cent. P_2O_5 , and muriate of potash equivalent to 4 per cent. K_2O . Uniform volumes were obtained by using appropriate quantities of gypsum as a filler.

Physiological studies

Green and dry weights.—One end of the plots on each section was selected for uniformity of stand, and from this end of each plot two hills were removed at 10 to 14 day intervals for determination of green and dry weights. The dried sample was stored for future analysis.

Height.—Early in the season four hills in each plot were marked with a garden stake and at each sample period the height of these hills was measured and averaged for the particular plot. In 1926 and 1927 all plants were measured to the tip of the inside leaf until the tassel appeared, then to the base of the tassel. It was hoped that such a measurement would give an index of vegetative height and eliminate reproductive growth and leaf length from the final measurements. However, the variations in height of the inside leaf were too great, and to correlate the results with other work at Wooster, all measurements were made to the top of the tallest leaf in subsequent years.

Leaf area.—Measurements of leaf area were made on the samples collected for green and dry weight determinations. When small, all six plants in the sample were measured; as they became larger, the number of plants on which leaf area measurements were made was reduced to expedite handling. When measurements were made on less than the total number of plants in the sample, leaf area was determined on those plants which closely approached the average field height for that sample date. All results were calculated to a single plant basis.

Measurement of leaf area was made on a specially constructed photometer involving photo-electric cells and vacuum tube amplification. A description of the photometer used in 1927 has been published (1). In 1928 it was found that the errors in the method could be reduced almost one-third by using two photo-electric cells in parallel.

Vein and intervein width.—In 1928 measurements were made to determine whether intervein width of the ear leaf might give an index of the efficiency of the leaf with respect to vegetative growth and yield. Measurements were made by an ocular and stage micrometer on a cross-section of the leaf taken at a definite distance above the leaf's junction with the stalk.

Stalk diameter.—Measurement of the least and greatest thickness of the stalks from the periodic samples was made at the center of the internode above the first node, and the two figures thus obtained were averaged and designated as stalk diameter. The average diameter of all six stalks in each periodic sample was then obtained.

Mean silking date.—Beginning with the appearance of the first silks, numbered tags were placed each morning on all ears which had silked over night. At the end of the silking period these tags were collected and the mean silking date calculated, (MEYERS, 5). Thus an index of the rate of

maturity was obtained as well as the effect of fertilizers on hastening the completion of this period in the life cycle of the plant.

Yield and moisture at harvest.—Harvest yields were determined on all plots and a 25-pound sample of ears selected at random. These samples were dried in a heated drying room and used to calculate the air-dry yields. Brown-Duvel moisture determinations were made on these samples and final yields calculated to a basis of 15.5 per cent. moisture. Marketable ears and nubbins were separated in the field at harvest, and, after drying, a shelling per cent. of the marketable ears was determined.

Results

EFFECT OF DEGREE OF FERTILITY UPON THE ACCUMULATION OF DRY WEIGHT

When the total dry weights from the periodic samples are plotted against time, the curves approach the S-shaped growth curve. However, as noted in figure 1 the curves for all plots receiving fertilizers exhibit two fairly well defined points of inflection. These curves would be of the bimodal type if plotted only on the actual increase in dry weight from sample to sample. Each curve apparently represents two S-shaped curves in which the autostatic end of the first curve overlaps the autokinetic end of the second (ROBERTSON, 7).

The type of curve presented in figure 1 may be correlated with the main phases of growth of the plant, vegetative and reproductive. The vegetative phase was practically complete on the fertilized plots at the mean date of silking. In every case, where large amounts of fertilizer were applied, the average date of silking occurred a few days after senescence became prominent in the vegetative phase. This was indicated by a slowing up of the growth rate and by the inflection in the curve of growth, or, in the words of ROBERTSON (7), "when vegetative growth attains maximal extent and minimal velocity."

From the graphs and tabular data (figures 1 and 2, and table I) it appears that hill application of fertilizer favors a more distinct separation of the vegetative and reproductive growth cycles. On the other hand, even much larger amounts of total nutrients supplied in broadcast applications of manure and superphosphate show but little of this tendency even though productive of higher final yields. Explanation apparently lies in the concentration of nutrients close to the seed in the hill treatments, this inducing more rapid early growth and earlier silking. Where hill treatment is combined with the broadcast application of manure and superphosphate, growth is rapid during both growth phases with greatest advance in silking date, and the two growth phases show distinct separation.

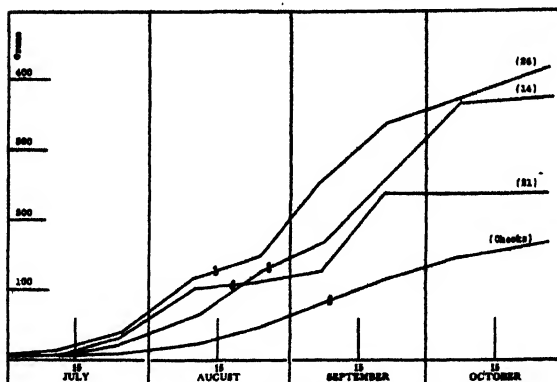


FIG. 1. Average dry weights of plants grown on plots receiving varying types of fertilizers. Planted May 15, 1927.

(26) Manure 8 tons, 0-16-0 225 lb. broadcast, 3-12-4 200 lb. hill.

(14) Manure 8 tons, 0-16-0 225 lb. broadcast.

(21) 3-12-4 200 lb. hill.

(Checks) average of all unfertilized plots.

S = mean silking date.

COMPARISON OF DRY WEIGHTS FOR 1926, 1927, AND 1928

The data so far presented apply only to the 1927 crop. The dry weight curves for 1926, 1927, and 1928 all follow the same general trends as shown in figure 3. Seasonal variation in meteorological conditions cause differ-

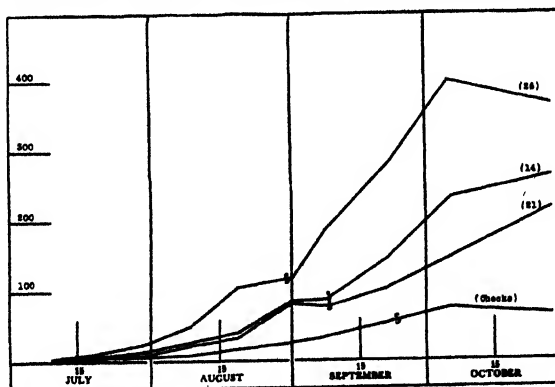


FIG. 2. Average dry weights of plants grown on plots receiving varying types of fertilizers. Planted June 5, 1927.

(26) Manure 8 tons, 0-16-0 225 lb. broadcast, 3-12-4 200 lb. hill.

(14) Manure 8 tons, 0-16-0 225 lb. broadcast.

(21) 3-12-4 200 lb. hill.

(Checks) average of all unfertilized plots.

S = mean silking date.

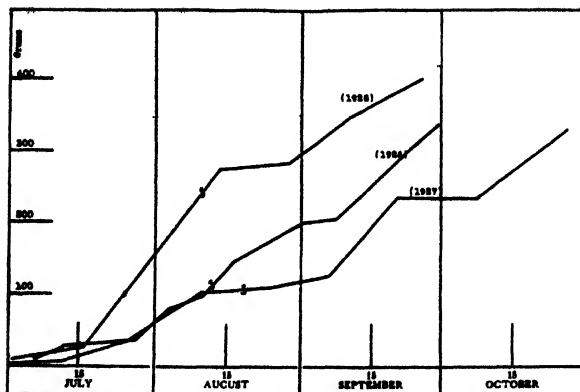


FIG. 3. Average dry weights of plants grown on all plots receiving hill applications of 3-12-4 in 1926, 1927, and 1928.

S = mean silking date.

ences in both the vegetative and reproductive phases of growth; hence, the averaging of data for two or more years would obscure the effects due to variations in climatic factors. The average date of silking varies by more than 6 days; evident senescence of vegetative growth varies by at least 20 days, while frost, sufficiently early and intense in 1926 and 1928 curtailed the reproductive growth sufficiently to prevent a complete expression of this portion of the cycle. The 1927 results, therefore, are more complete and were selected to indicate the general trends of growth as shown by dry weight accumulation.

The effects of variations from the normal in temperature and rainfall throughout the growing season on increase in dry matter are presented in figure 4. In 1928 an extremely wet and cold June resulted in the storage of considerable moisture in the soil. July was warm with about normal precipitation; as a result, a large quantity of nitrate nitrogen was probably produced in the soil. These favorable conditions of moisture, temperature, and available nitrogen were directly reflected in the rapid growth at this time, as contrasted with that during the same period in 1927 which was undoubtedly inhibited as the result of cold, dry weather in June. July of 1927 was about normal but not sufficiently favorable to make up for the June deficiency in temperature and rainfall. In 1928 an extremely warm August with more than normal precipitation provided conditions favorable to rapid growth, while the opposite seasonal conditions occurred in 1927. This causes the dry weight curves for these two years to diverge widely at this time. However, an early frost and a cold dry autumn stopped all growth before the end of September in 1928, while more favorable conditions permitted the corn to grow at least thirty days longer in 1927.

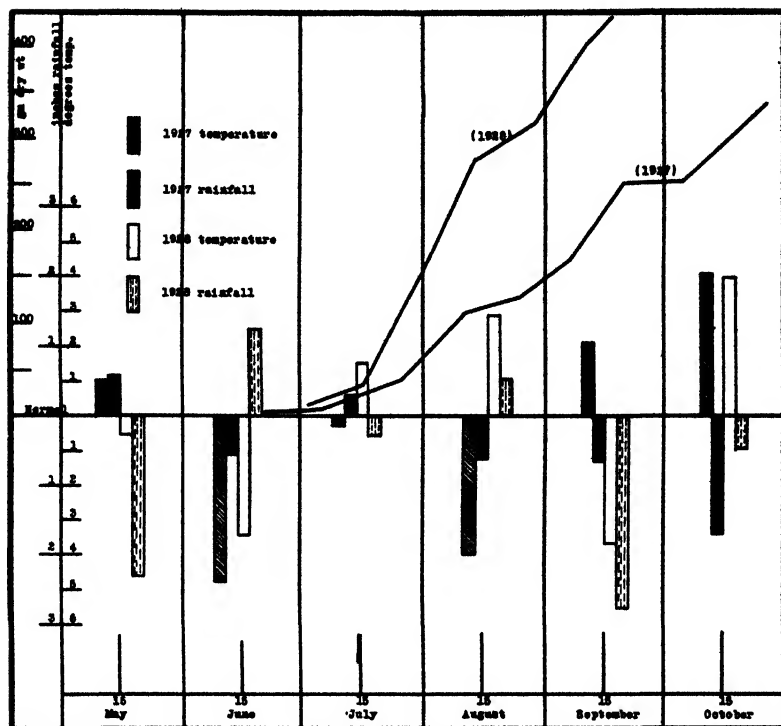


FIG. 4. Correlation of dry weight increase with seasonal variation in climatic factors. Average of all plots receiving hill applications of 3-12-4.

EFFECT OF DEGREE OF FERTILITY ON STALK DIAMETER, LEAF AREA, HEIGHT, AND SILKING DATE

Figure 5 shows the average stalk diameter of corn at different degrees of fertility. The most interesting observation is the closeness of the maximum diameter to a single value for all three plots, indicating that Burr-Leaming corn has a seasonal maximum diameter which it reaches with comparatively low fertility. The higher the degree of fertility, the more rapid was the early growth and the attainment of this maximum stalk diameter. The fact that Burr-Leaming corn attains a seasonal maximum diameter at comparatively low levels of fertility is further indicated in figure 9 in which the average maximum diameter for 1927 and 1928 from twelve different plots is plotted against the yield produced at increasing degrees of fertility.

The leaf area curves for the plots receiving 3-12-4 fertilizer in the hill in 1926, 1927, and 1928 are presented in figure 6. The most striking thing about these curves is the rapid acceleration in leaf area development during

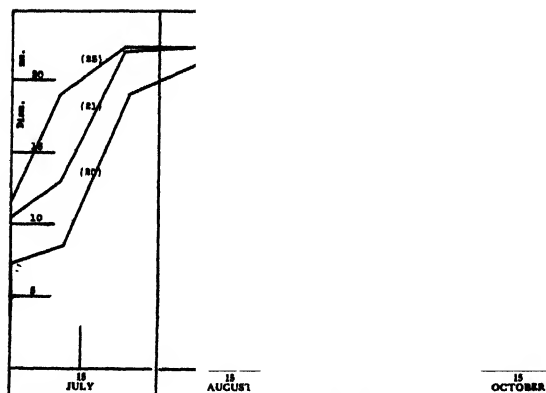


FIG. 5. Average stalk diameter of plants grown on plots receiving varying types of fertilizers. Planted May 15, 1927.

(23) 3-12-4 400 lb. hill.

(21) 3-12-4 200 lb. hill.

(20) 3-12-4 100 lb. hill.

the early part of 1928. There may have been an initial lag in the acceleration, but if so it was too small to be measured within the sample periods. However, though acceleration is rapid in the early stages and the maximum area attained is greater than in previous years, a longer time is required to reach this maximum leaf area.

The 1927 data are used for comparing the effects of fertility treatments upon increase of leaf area, the effects of manure and superphosphate alone, complete fertilizer alone, and the combination of these two fertilizers being shown in figure 7.

It is apparent that a seasonal maximum leaf area may be obtained by the use of fertilizers. This limitation of leaf area is similar to that observed for stalk diameter. The three early planted, fertilized plots shown in figure 7 represent a wide variation in fertility; yet the maximum leaf area attained by any one of these plots varies by less than 200 square centimeters from that of either of the others. The maximum variation between the fertilized plots is approximately 4.5 per cent. while any one of these three plots varies from the unfertilized check plot by 2,000 cm.², or 45 per cent. Further evidence of the production of maximum leaf area at relatively low degrees of fertility is shown in figure 9, in which the two year average maximum leaf area is compared with the yield at increasing levels of fertility.

From observation of figure 1 it is noted that the increase in dry weight for these plots is apparently independent of either leaf area or stalk diameter. This may indicate that the nutrients are first utilized in the

growth of the stalk and leaves up to a certain size. If these nutrients are just sufficient to permit maximum attainment of vegetative growth for a given season, little is left for reproductive growth. Where fertilizers are added to the soil and absorbed in excess of the requirements for vegetative processes, the excess becomes available for continued reproductive growth. This relationship is further indicated by the data presented in table II, showing the leaf areas and yields at several degrees of fertility for the years 1927 and 1928.

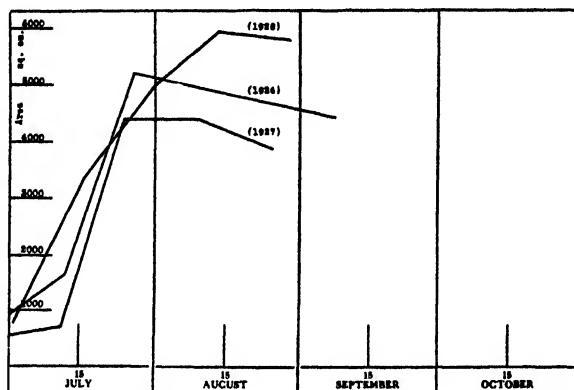


FIG. 6. Average leaf area of plants grown on all plots receiving hill applications of 3-12-4 in 1926, 1927, and 1928. Planted about May 15, each year.

In the analysis of these data it must be remembered that a single leaf may have an area of 200 to 300 cm.² or even more. The loss of a single leaf, due to drying and breaking off, would affect the leaf area of the plants sampled by a considerable amount. This loss of leaves occurring earlier in the early planted corn accounts at least in part for the greater area of leaves in the last samples of late planted, heavily fertilized corn. However, the data as a whole substantiate the previous hypothesis that for Burr-Leaming corn a maximum leaf area is attained at fairly low concentrations of fertilizer.

The maximum leaf area attainable in any season is determined in part by meteorological conditions, such as rainfall and temperature. For the years 1926 and 1927 the maximum area reached varied between 4500 and 5000 cm.²; while for the year 1928 it was 6500 to 7000 cm.² The latter year was characterized by heavy rainfall and high temperatures. However, the relative effects of fertilizers upon attainment of maximum leaf area for the season was about the same in all three years, a fairly low degree of fertility being capable of producing a maximum leaf area equal to that attained by the heaviest fertilized corn.

Apparently, from a study of the data in tables I and II and the figures 7, 8 and 9, neither maximum leaf area nor time of its attainment are indices of potential yield for every degree of fertility. Below that degree of fertility at which maximum leaf area is attained, yield and leaf area decrease

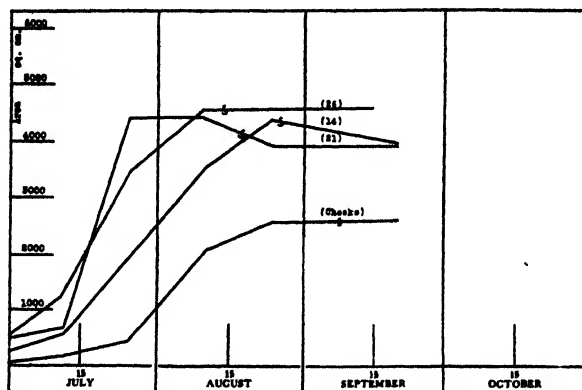


FIG. 7. Average leaf area of plants grown on plots receiving varying types of fertilizers. Planted May 15, 1927.

(26) Manure 8 tons, 0-16-0 225 lb. broadcast, 3-12-4 200 lb. hill.

(14) Manure 8 tons, 0-16-0 225 lb. broadcast.

(21) 3-12-4 200 lb. hill.

(Checks) average of all unfertilized plots.

S = mean silking date.

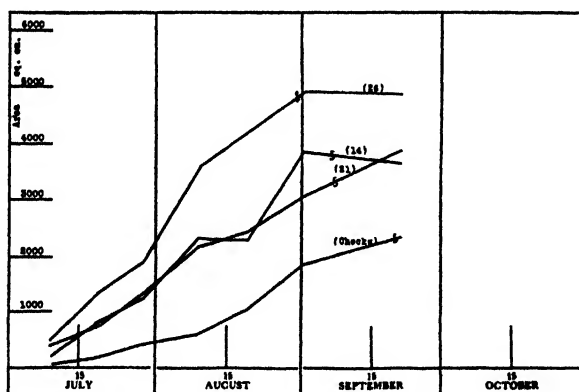


FIG. 8. Average leaf area of plants grown on plots receiving varying types of fertilizers. Planted June 5, 1927.

(26) Manure 8 tons, 0-16-0 225 lb. broadcast, 3-12-4 200 lb. hill.

(14) Manure 8 tons, 0-16-0 225 lb. broadcast.

(21) 3-12-4 200 lb. hill.

(Checks) average of all unfertilized plots.

S = mean silking date.

almost proportionally; above this degree of fertility, yield increases very rapidly while leaf area remains constant.

TABLE I

YIELD OF CORN AS AFFECTED BY DEGREE OF FERTILITY

PLOT	TREATMENT	PLANTED MAY 15, 1927			PLANTED JUNE 5, 1927		
		YIELD			YIELD		
		EARS	NUBS	TOTAL	EARS	NUBS	TOTAL
26	manure, 8 T., 0-16-0 225 lb. Bc., 3-12-4 200 lb. hill	bu.	bu.	bu.	bu.	bu.	bu.
		55.6	14.7	70.3	39.5	8.4	47.9
14	manure, 8 T., 0-16-0 225 lb. Bc.	27.9	17.3	45.2	14.3	17.6	31.9
21	3-12-4 200 lb. hill	23.8	13.4	37.2	11.4	13.2	24.6
Checks	unfertilized	4.7	12.5	17.2	1.3	3.5	4.8

In plotting the curves in figure 9 the fertilized plots were arranged in order of yield, and not always in order of increasing fertilizer application. Often the method of application was just as effective in determining the fertility level and yield on the plots as the quantity applied.

The vein and intervein widths of ten leaves removed at the ear node from each of three different plots were measured at a point 10 cm. above the junction of the leaf with the ear. The results are presented in table III.

Although there is a slightly greater intervein width in the leaves of corn from highly fertilized plots, the difference is so small as to be practically negligible when contrasted with the great differences in yield.

Since seasonal maximum leaf area is reached at relatively low fertility; and, as there is almost no increase in the intervein vein ratio, it would appear that the attainment of the maximum efficiency of the leaves of Burr-Leaming corn is limited by the nutrients available in the soil.

The general trend of increase in height throughout the growing season is affected by fertility in a manner quite similar to either dry weight, leaf area, or stalk diameter. (See figures 9, 10, and 11.) However, the final height apparently is not so much affected by internal physiological conditions as are leaf area and stalk diameter; that is, though there is probably a genetic and physiological limitation to final height beyond which the degree of fertility will not produce greater elongation of the stem, this limitation

TABLE II
LEAF AREA AND YIELD OF THE CORN PLANT AS AFFECTED BY THE DEGREE OF FERTILITY

TREATMENT	PLANTED MAY 15					PLANTED JUNE 5				
	MAXIMUM LEAF AREA			YIELD		MAXIMUM LEAF AREA			YIELD	
	1927	1928	Av.	1927	1928	1927	1928	Av.	1927	1928
Manure, 8 T. } broadcast 0-16-0 225 lbs. } 3-12-4 200 lb. hill	cm. ² 4510	cm. ² 7032	cm. ² 5771	bu. 70.3	bu. 85.2	cm. ² 4960	cm. ² 7965	cm. ² 6462	bu. 47.9	bu. 80.5*
Manure, 8 T. } broadcast 0-16-0 225 lb. }	4343	6953	5648	45.2	75.0	3877	6520	5198	31.9	29.7*
3-12-4 400 lb. hill	4565	6970	5768	44.2	63.7	5057	7259	6158	25.1	44.0
3-12-4 200 lb. hill	4484	5917	5151	37.2	63.5	3820	6615	5217	24.6	48.2
3-12-4 100 lb. hill	3678	5232	4455	27.9	66.5*	3180	5965	4572	19.6	45.0
Checks, unfertilized	2833	4770	3801	20.3	43.1	2317	5234	3775	6.7	32.1

* Adjacent check yielded 55.2 bushels per acre.

+ Adjacent check yielded 13.7 bushels per acre.

* Yield calculated from less than total plot area after damage by birds.

TABLE III
VEIN AND INTERVEIN WIDTH OF CORN LEAVES

TREATMENT	WIDTH		RATIO
	AV. OF TEN LEAVES		INTERVEIN
	VEIN	INTERVEIN	VEIN
Manure, 8 T., 0-16-0 225 lb. broadcast	mm.	mm.	
3-12-4 200 lb. hill	0.10166	101.2	1/995
3-12-4 200 lb. hill	0.09243	91.4	1/988
Checks, unfertilized	0.08466	81.7	1/965

is not effective at such low fertility levels as seemed to cause the expression of the internal limiting factors for leaf area or stalk diameter.

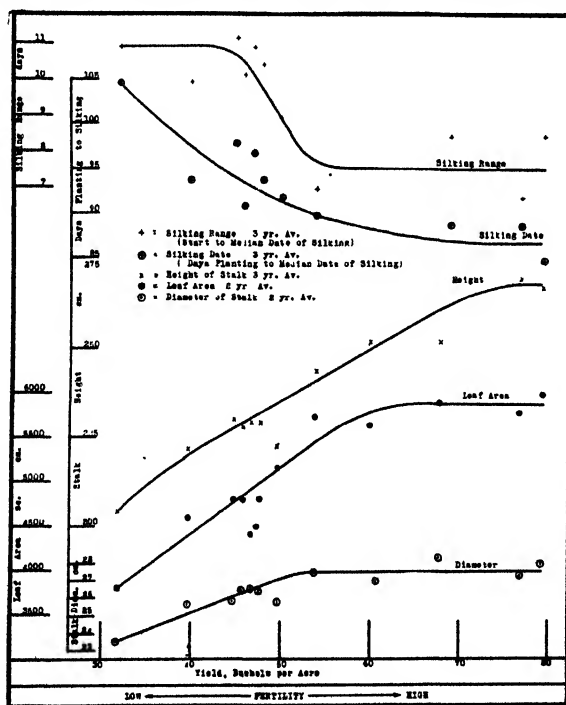


FIG. 9. Effect of degree of fertility on the yield and physiological development of the corn plant.

The failure of the lower fertilized plots to reach heights even approximately equal to those of the higher fertilized plots is pronounced, as is

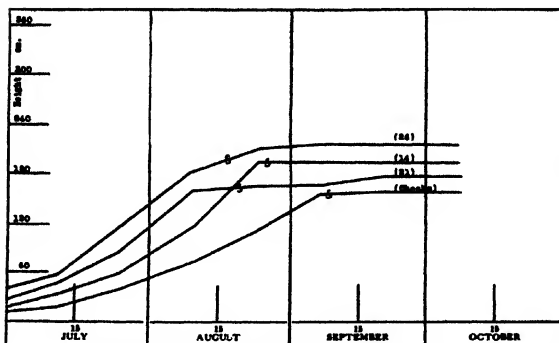


FIG. 10. Average height of plants grown on plots receiving varying types of fertilizers. Planted May 15, 1927.

(26) Manure 8 tons, 0-16-0 225 lb. broadcast, 3-12-4 200 lb. hill.

(14) Manure 8 tons, 0-16-0 225 lb. broadcast.

(21) 3-12-4 200 lb. hill.

(Checks) average of all unfertilized plots.

S = mean silking date.

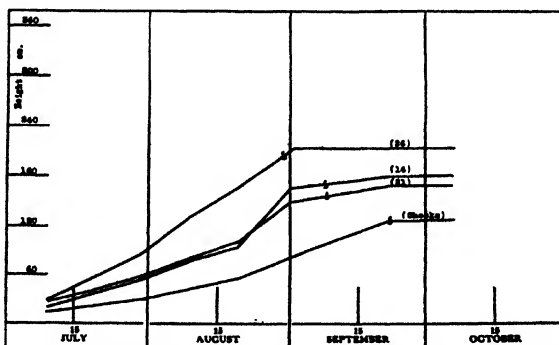


FIG. 11. Average height of plants grown on plots receiving varying types of fertilizers. Planted June 5, 1927.

(26) Manure 8 tons, 0-16-0 225 lb. broadcast, 3-12-4 200 lb. hill.

(14) Manure 8 tons, 0-16-0 225 lb. broadcast.

(21) 3-12-4 200 lb. hill.

(Checks) average of all unfertilized plots.

S = mean silking date.

also the fact that the highly fertilized plots for both early and late plantings produce corn of nearly equal height. This would indicate that this height of approximately 225 centimeters is probably the limit of elongation for that particular year. Further evidence of the fact that the maxi-

imum potentiality for height is expressed only at high degrees of fertility is given in table IV. The fact that height is limited only at high levels of

TABLE IV
HEIGHT OF THE CORN PLANT AS AFFECTED BY THE DEGREE OF FERTILITY

TREATMENT	FINAL HEIGHT			
	1927*	1928*	1929*	Av.
Manure, 8 T., 0-16-0 225 lb. broadcast 3-12-4 400 lb. hill	cm. 225	cm. 318	cm. 260	cm. 267.6
Manure, 8 T., 0-16-0 225 lb. broadcast 3-12-4 200 lb. hill	215	330	265	270.0
Manure, 8 T., 0-16-0 225 lb. broadcast 3-12-4 200 lb. hill	195	308	255	252.6
	170	265	227	221.0

* 1927 height measured to base of tassel.

* 1928 and 1929 height measured to top of tassel.

fertility is shown in figure 9 in which the three year average maximum height is compared with yield for increasing degrees of fertility.

The value of the date of silking as an index of various physiological phenomena can scarcely be over-emphasized. The method of obtaining the mean date of silking has been previously discussed by MEYERS (5).

The mean silking date occurs almost always at the time when maximum vegetative growth is attained, as shown in all of the previous graphs. In some cases, this point falls slightly ahead of the maximum attainment of height or leaf area, in others it occurs after these maxima have been reached. Photoperiodism is almost certain to play an important rôle in the determination of the time of silking, but it cannot completely overcome the handicap of poor environment, late planting, and lack of varietal adaptation.

Although photoperiodism may play an important part in the determination of the flowering time of plants, in the corn plant silking exhibits sufficient variability in time of occurrence to be influenced by fertility to an extent which is not only measurable but of economic importance (tables V and VI).

The most notable effects were produced by hill applications of complete fertilizer supplementing broadcast applications of manure and superphosphate. Such treatment not only caused earlier silking but also reduced the length of the silking period. In 1927 the plot receiving this treatment reached the mean date of silking 24 days earlier and completed the silking

TABLE V
AVERAGE SILKING DATE AND YIELD OF CORN PLANT AS AFFECTED BY DEGREE OF FERTILITY. PLANTED MAY 15

TREATMENT	PLANTING TO SILKING		SILKING PERIOD*	YIELD, 15.5 PER CENT. MOISTURE				MOISTURE AT HUSKING			
	1927	3 YEAR AV.		1927	1927		3 YEAR AV.	1927	3 YEAR AV.		
					MARKET- ABLE	TOTAL				MARKET- ABLE	TOTAL
Manure, 8 T. } broadcast											
0-16-0 225 lb. } hill	92	89	7	55.6	70.3	bu.	68.8	bu.	77.7		
3-12-4 200 lb. } hill	104	98	11	27.9	45.2	bu.	51.8	bu.	65.5		
Manure, 8 T. } broadcast											
0-16-0 225 lb. } hill	93	90	7	30.4	41.2	bu.	47.8	bu.	58.4		
3-12-4 400 lb. } hill	97	92	9	23.8	37.2	bu.	43.5	bu.	55.1		
3-12-4 200 lb. } hill	104	97	11	14.2	27.9	bu.	41.8	bu.	52.8		
3-12-4 100 lb. } hill	116	105	11	6.5	20.3	bu.	24.7	bu.	39.2		
Checks unfertilized											
								per cent.	per cent.		
								33.6	35.1		
								46.7	42.0		
								36.9	36.8		
								36.9	36.7		
								41.9	40.0		
								46.7	45.1		

* Number of days from start of silking to average date of silking.

TABLE VI
AVERAGE SILKING DATE AND YIELD OF CORN PLANT AS AFFECTED BY DEGREE OF FERTILITY. PLANTED JUNE 5

TREATMENT	PLANTING TO SILKING		SILKING PERIOD*	YIELD, 15.5 PER CENT. MOISTURE				MOISTURE AT HUSKING	
	1927	3 YEAR AV.		1927		3 YEAR AV.		1927	3 YEAR AV.
			MARKET-ABLE	TOTAL	MARKET-ABLE	TOTAL	per cent.		
Manure, 8 T. } broadcast 0-16-0 225 lb. } 3-12-4 200 lb. hill Manure, 8 T. } broadcast 0-16-0 225 lb. } 3-12-4 400 lb. hill 3-12-4 200 lb. hill 3-12-4 100 lb. hill Checks unfertilized	days	days	1927	MARKET-ABLE	TOTAL	MARKET-ABLE	TOTAL	per cent.	per cent.
		88	11	bu.	47.9	bu.	bu.	50.1	55.0
	78			39.5			36.9	53.5	48.4
				14.3	31.9	20.9	42.3	42.6	43.6
	87	83	10	13.2	22.9	22.4	26.1	54.3	49.0
	84	82	10	11.4	24.6	26.1	34.9	67.5	58.2
	87	84	10	7.2	19.6	24.1	28.5		
	90	86	9	1.8	6.7	16.1			
	99	93	9						

* Number of days from start of silking to average date of silking.
Three year average unavailable for first plot in this table.

period in 8 days less time than the unfertilized plots. Further comparison of the data shows that as the quantity of chemical fertilizer is decreased the time to reach the average date of silking becomes greater and the time for completion of silking is increased.

The importance of method of application of nutrients, in this connection, is shown by the results for the plot receiving manure and superphosphate without supplementary hill treatment. On the basis of its yield and of the results obtained by WOLFE (9) previously discussed, this plot should silk earlier than any of the plots receiving only complete fertilizer. In all other cases the higher the yield, the earlier occurred the mean date of silking. The failure of this plot to silk earlier may be accounted for by the slow initial growth as demonstrated by the leaf area, height, and dry weight curves for this treatment. It is also evident from the data in tables V and VI that late silking is correlated with high moisture content in the grain at harvest, a factor of considerable economic importance since moisture content is the most important factor determining the grade of market corn.

The effect of fertility upon the rate and duration of silking is presented graphically in figure 12. The rapidity of silk development with high fertility and the slow development and the long drawn-out blooming period with low fertility are emphasized in this graph.

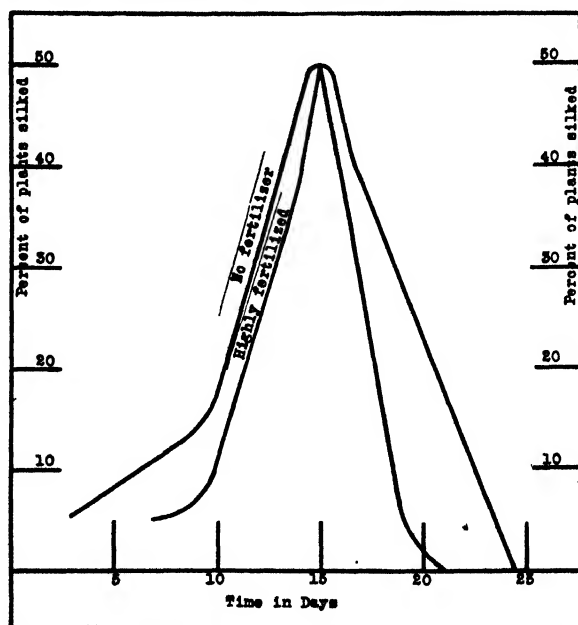


FIG. 12. Rate of silking as affected by fertility.

That earliness of silking and shortening of the duration of the silking period are limited by fertility level as are leaf area, height and diameter is shown in figure 9.

Low concentrations of fertilizer are not effective in shortening the silking period, but at medium concentrations the duration of the silking period is rapidly shortened to the minimum attainable with Burr-Leaming corn. The earliest median date of silking, however, is reached only at relatively high levels of fertility.

Probably the silking phenomena are greatly influenced by the response of individuals in the heterogeneous population of these plots. Low fertility levels appear to affect the silking phenomena in a manner similar to dry weight accumulation, for those plots which exhibit the longest duration of the silking period, and which reach the average date of silking late in the season, also exhibit the most complete mergence of the growth and reproductive cycles as shown by the smooth, single sigmoid curve for dry weight accumulation.

A conclusion of considerable practical value that may be drawn from a study of the data for silking date, yield, and moisture at harvest is the possibility of maturing an otherwise unadapted, long-season variety of corn by the use of sufficient fertilizer applied in the hill.

Summary

Periodic measurements of the physiological development of the corn plant were made on corn grown on plots representing 19 different degrees of fertility and two different dates of planting.

Periodic measurements on samples collected at ten- to fourteen-day intervals included green weight, dry weight, stalk diameter, and leaf area.

Field measurements included height, silking date, and yield of grain. The moisture content of the grain at harvest was also determined.

A summary of the results follows:

1. With continuous abundance of nitrogen, phosphorus, and potassium throughout the season, marked differentiation of the vegetative and reproductive cycles occurred, associated with a narrow silking range, earliness of silking, and high yield.

2. With a low supply of nitrogen, phosphorus, and potassium throughout the season, an overlapping and mergence of the two growth cycles occurred, associated with a wide silking range, lateness of silking, and low yield.

3. Hill applications of fertilizers appeared to intensify the differentiation of the vegetative and reproductive cycles. Broadcast applications were markedly less effective, presumably because less nutrients were concentrated within reach of the plant during its early period of growth.

4. Maximum leaf area, stalk diameter, and height of the corn plant were produced at levels of fertility considerably below that which still produced increase in yield.

5. The narrowest silking range and earliest average date of silking occurred at levels of fertility considerably lower than that which still produced increase in yield.

6. The degree of fertility at which the different physiological maxima were obtained was approximately the same from year to year, regardless of the annual fluctuation in maxima due to seasonal conditions.

7. Variations in the physiological development of corn from different levels of fertility may represent, under the conditions of this experiment, a variable response of individuals in the heterogeneous population of Burr-Leaming corn.

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COMPARISON OF TWO METHODS OF MEASURING STOMATAL APERTURE

ERIC ASHBY¹

(WITH ONE FIGURE)

Both England and America have contributed valuable data to our knowledge of stomatal movement in plants; and it is of interest that totally different methods have been used in the two countries. In England the porometer, first described by DARWIN and PERTZ (2) was modified and improved by KNIGHT (4), who used the method for his work on stomatal movement (5, 6). In the United States the most important contributions have been made by LLOYD (7), E. B. SHREVE (9), and LOFTFIELD (8). LLOYD originated the method of tearing off the epidermis, and plunging it immediately into absolute alcohol, subsequently measuring the stomatal aperture under the microscope. This method was also adopted by LOFTFIELD. SHREVE described a method depending upon rapid penetration by picric acid, and subsequent microscopic examination.

KNIGHT, though he mentions LLOYD's method, has published no comparison of the method with his own. LOFTFIELD rejects the porometer method. "To the best of the writer's knowledge, no comparison has been made between a series of direct observations upon the condition of the stomata and porometer readings made at the same time. Until the method is checked in this manner it is felt that its reliability is questionable." (8, page 8.)

Both LLOYD's method and KNIGHT's have their draw-backs, which it is not the writer's purpose to discuss here. A brief comparison of the two methods may not be out of place, especially as the British method could be applied on the same species as LLOYD used, and in the same laboratory as that in which LLOYD devised his method.

For the tests two plants were used, geranium, growing in the greenhouse of the desert laboratory, and *Verbena*, growing in the garden. A porometer, similar to that described by KNIGHT (4), was fixed on one leaf of the plant. Readings were taken on the porometer at regular intervals. Every time a reading was taken, a strip of epidermis was removed from a leaf of about the same age, and put into a solution of congo red in absolute alcohol. The dimensions were measured under the microscope, and the areas of the pores calculated, on the assumption that the pores were elliptical in outline. In table I, and figure 1 are given the readings from

¹ Commonwealth Fund Fellow.

one experiment on a geranium leaf, in full, and in table II are readings in full made on a leaf of *Verbena*.

It will be noticed that the porometer readings are compared with the areas of the pores, and not the diameters. While diffusion is proportional to the diameters of the pores, the movement of gas under pressure is proportional to the areas.

The tables reveal certain facts relating to the relative sensitiveness of the two methods. LLOYD himself mentions that "the stomata in a piece of epidermis are seldom of a uniform degree of opening." (7, page 28.); but his method of estimating the mean stomatal aperture by measuring the extremes of aperture is not statistically sound. When the mean of ten stomatal apertures is taken, the coefficients of variability are of the order of 20 per cent. The porometer averages the apertures of some 50,000 stomata, and the coefficient of variability of five readings is as low as 3.2 per cent. Assuming that it measures stomatal aperture, the porometer, then, is a far more sensitive test of changes of aperture than LLOYD's method. It suffers, however, from the disadvantage that absolute values cannot be obtained from the method.

TABLE I
STOMATAL MOVEMENT ON GERANIUM LEAF THROUGHOUT THE DAY

TIME	POROMETER METHOD	LLOYD'S METHOD, AREA OF PORE
	<i>drops per minute</i>	<i>mm.²</i>
9:30 A. M.	porometer put on	
10:00	7.6 \pm 0.24	
11:00	8.1 \pm 0.26	0.000118 \pm 10 ⁻⁵ \times 2.17
12:00	8.0	
1:00 P. M.	7.8 \pm 0.26	0.000116 \pm 10 ⁻⁵ \times 2.47
2:00	7.4 \pm 0.22	0.000095 \pm 10 ⁻⁵ \times 1.82
3:00	6.4 \pm 0.20	0.000086 \pm 10 ⁻⁵ \times 1.70
4:00	6.9 \pm 0.20	0.000077 \pm 10 ⁻⁵ \times 1.15
5:00	6.0 \pm 0.19	0.000079 \pm 10 ⁻⁵ \times 2.49
6:00	2.8 \pm 0.02	0.000046 \pm 10 ⁻⁵ \times 0.73
7:00	2.0 \pm 0.02	0.000000

To test the closeness of agreement of the two methods, the two sets of readings are arranged as percentages of their maximum values, and the series of differences between the percentages compared, using the statistic χ^2 , and the tables in FISHER's monograph (3). This has been done in full for the geranium leaf (table III), and in table IV are given the results of tests on various other leaves.

TABLE II
STOMATAL MOVEMENT ON *Verbena* LEAF THROUGHOUT THE DAY

TIME	POROMETER	LLOYD'S METHOD
	<i>drops per minute</i>	<i>mm.²</i>
9:30 A. M.	porometer put on
11:00 A. M.	4.8	$0.000090 \pm 10^{-4} \times 1.8$
12:00	4.5	$0.000082 \pm 10^{-4} \times 1.6$
3:00 P. M.	2.0	$0.000048 \pm 10^{-4} \times 0.9$
6:00 P. M.	1.1	$0.000015 \pm 10^{-4} \times 0.7$

It is clear that the methods do not give results which differ significantly, except at very small stomatal apertures. At 7:00 P. M. the stomata appeared closed when examined by LLOYD'S method under the microscope, but the porometer still gave readings. This was almost invariably observed. It is not surprising that air can still be drawn through the stomata slowly after they appear to be closed. At such apertures, however, diffusion through the pores is proportional to the cube or sixth root of the rate of flow under pressure, so that LLOYD'S method gives the more accurate picture of the diffusive capacity of stomata at small apertures.

TABLE III
 χ^2 TEST OF DIFFERENCES BETWEEN THE TWO METHODS ON GERANIUM

TIME	POROMETER READING IN PERCENTAGE OF MAXIMUM	LLOYD'S METHOD IN PERCENTAGE OF MAXIMUM	DIFFER- ENCE	χ^2
	<i>per cent.</i>	<i>per cent.</i>		
11:00	100	100	0	0
1:00	96.3	98.0	1.7	0
2:00	91.4	81.0	10.4	0.62
3:00	79.0	73.1	5.9	0.48
4:00	85.2	65.0	20.2	6.28
5:00	74.1	66.2	7.9	0.94
6:00	34.7	39.3	4.6	0.54
7:00	24.7	00.0	24.7	(610.1)

Total, omitting last fig. = 8.86

n = 5

P = 0.10-0.20

The tables reveal a very satisfactory agreement between KNIGHT'S method and LLOYD'S method of estimating stomatal apertures, on geranium and *Verbena*, at wide apertures. If the small values, taken about sunset,

TABLE IV
SUMMARY OF TESTS OF AGREEMENT BETWEEN THE TWO METHODS

NUMBER	PLANT	χ^2	n	P	DIFFERENCE
1	<i>Geranium</i>	8.86	5	0.10-0.20	not significant
2	"	7.68	4	0.10	" "
3	<i>Verbena</i>	5.40	4	0.30-0.20	" "
4	"	9.00	5	0.10	" "
5	"	5.02	4	0.30-0.20	" "

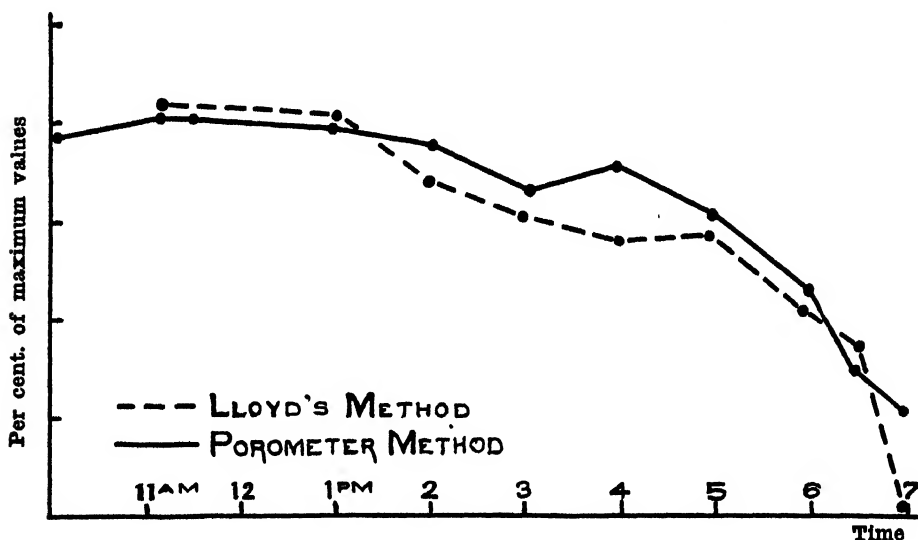


FIG. 1. Diurnal changes in stomatal aperture of the geranium, determined by two different methods.

are included, the values of χ^2 are 618.9, 442.3, 330.2, instead of 8.86, 7.68, and 2.29 and the divergence is in every case significant. Neither of these plants has heterobaric leaves.

According to the coefficients of variability of the two methods, the porometer method is nearly ten times as sensitive as the method devised by LLOYD.

In conclusion I wish to record my indebtedness to Dr. SHREVE, and my thanks to the Carnegie Institution for the facilities of the Desert Laboratory.

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MECHANISM OF ROOT-CONTRACTION IN *BRODIAEA* *LACTEA*

D. THODAY

In a recent paper in the American Journal of Botany, F. H. SMITH (7) has given an interesting account of the contractile roots of *Brodiaea lactea*. These show features that clearly differentiate them from the ordinary monocotyledonous types of contractile root which the work of RIMBACH (4, 5, 6) has made familiar. In the latter types the stele remains straight, owing to the fact that the taut contractile tissue lies just outside it. The cells of this active tissue, the inner cortex, grow wider and shorter, as described by DE VRIES for other types (13). Room for their transverse growth is obtained in many cases by progressive collapse of the outer cortical cells. In *Brodiaea lactea* the outer cortex does not collapse in this way and in the later stages of contraction the stele becomes greatly distorted, which shows that the tissue immediately surrounding it cannot be actively contracting and so longitudinally taut. According to SMITH's account it seems that there may be two phases of contraction, the first, during which the stele remains straight, brought about in the ordinary way by growth contraction of the cortex while the root is still swelling; the second, after swelling is complete, involving the distortion of the stele and progressive *collapse of transverse zones* of the cortex.

The alternation of turgid with collapsing transverse zones parallels in so remarkable a way the phenomena described by the writer for the Dicotyledon, *Oxalis incarnata* (9), that it seems worth while to draw attention to the similarity and to suggest that the explanation offered of the mechanism of the one may be applicable to the other.

SMITH's explanation of the later phase of contraction is not convincing. He appears to attribute it to the distortion of the stele in consequence of the unequal distribution around it of turgid growing cells exerting on it unequal lateral pulls. The turgid layers, owing to the loss of turgor of layers above and below, are released from vertical pressure, round off and so diminish their transverse dimensions and pull on the stele. If this were the explanation the stele would be under longitudinal tension. Transverse components of this tension would be borne by the turgid tissue pulling on the convexities of the stele. If, however, the stele is compressed during the first phase of contraction, it is less likely to offer resistance to extension. Besides, a glance at the violence of its distortion makes it impossible to regard it as a taut cord. SMITH refers to the tearing of the tissue just out-

side the stele and states that it is mostly the collapsed cells that are torn loose. His fig. 7, however, shows that tearing is not confined to them.

The salient facts for an understanding of the mechanics of the situation are the following: (1) *The stele is being distorted under longitudinal compression*: the tearing of the inner cortex seems adequately accounted for as consequential upon this distortion. (2) The turgid transverse layers "round off again, increasing somewhat in length and decreasing in their radial and tangential measurements. Thus from this stage on, as the root contracts, the diameter of the root as a whole decreases." That is, *the volume of the root is diminishing* by longitudinal and to a smaller extent by transverse contraction, so that *sap must be passing out* of it—mainly it is clear, *from the collapsing cells*.

The parallel with *Oxalis incarnata* is very close. In both cases the contraction overcomes the resistance of a central core, including lignified elements, which yields by violent distortion. In neither can any tissue be found which has the continuity essential to an active contractile tissue, which must be capable itself of bearing longitudinal strain. In both cases transverse zones of collapsing tissue determine that the diminution of volume shall affect mainly the length of the organ, while intervening turgid zones prevent transverse collapse.

The explanation of the contraction thus turns on the evacuation of the collapsing cells. (Cf. 9, pp. 575–576.) If these cells lose their protoplasm, as they do in *Oxalis incarnata*, and no longer retain their solutes, the existence of a water-deficit elsewhere in the plant will lead to a withdrawal of their water. In a transpiring plant this is the normal condition. Very rarely can the cells of land plants be fully turgid. The maintenance of the transpiration stream ordinarily involves the maintenance of a gradient of water deficit and a gradient of suction pressure (1, 3, 7, 10, 11, 12).

If now we imagine that a living cell in equilibrium with its neighbors, *i.e.* with equal suction pressure (10), is replaced by a cell-wall full of pure water, the equilibrium is disturbed. The wall of this cell will not be osmotically distended; the osmotic pressure of the contained liquid is nil; the suction pressure, the measure of its power to absorb or retain water, is zero. It will yield water, therefore, to the neighboring cells. The limiting conditions can be best understood by considering first a simplified case of one such dead cell in contact with one living cell. (We will suppose that they are protected from evaporation.)

Directly or indirectly, the pressure of the atmosphere acts upon them. The pressures are therefore

in the living cell, l:

osmotic pressure of cell sap, P_1 ;

hydrostatic pressure of cell sap, H_1 , which balances the sum of the

wall pressure, W_1 , and
atmospheric pressure, A .

in the dead cell, d :

osmotic pressure of the liquid, $P_d = 0$
hydrostatic pressure, $H_d = A$, since
wall pressure, $W_d = 0$.

The pressure determining the passage of water from the dead into the living cell will be the difference between $P_1 - H_1 = P_1 - W_1 - A$ and $P_d - H_d = 0 - A$, *i.e.* $P_1 - W_1$, which is the suction pressure of the living cell, as ordinarily defined (S_1).

Water will be withdrawn, therefore, till $W_1 = P_1$, *i.e.* till the living cell is fully turgid, unless resistance is offered to the consequent diminution of its volume by the wall of the dead cell. In the latter case the diminishing volume of water pulls upon the walls. Strictly speaking, the first effect is a reduction of the hydrostatic pressure of the water below atmospheric pressure, the water menisci are pushed back into the wall by the atmospheric pressure, an increasing share of which is, therefore, borne by the wall. If the wall were rigid it would come ultimately to support the whole of the atmospheric pressure acting on the outside of the cell. In the dead cell we should then have $H_d = 0$; $W_d = -A$.

The pressure determining movement of water would be the difference between $P_1 - W_1 - A$, and $P_d - H_d = 0$; *i.e.* $P_1 - W_1 - A$, or $S_1 - A$.

If the suction pressure of the living cell were greater than one atmosphere the removal of water from the dead cell would continue, increasing the strain on the wall and bringing the water in it into a state of tension, so that $W_d = -A + H_d$ where H_d is negative. Call the cohesion tension $C_d = -H_d$.

For equilibrium now between living and dead cells $P_1 - H_1 = P_d - H_d$, *i.e.* $P_1 - W_1 - A = 0 - H_d = C_d$, or $S_1 = C_d + A$.

The significance of this is, that if a cell loses protoplasm and solutes it will retain its water and resist collapse only if its wall is capable of withstanding a pressure difference, acting inwards, equivalent to the suction pressure of the neighboring cells. Actually, this pressure, up to the value of an atmosphere, is the external pressure of the atmosphere unbalanced by an equivalent hydrostatic pressure within the cell; beyond one atmosphere it is atmospheric pressure acting outside together with a cohesion tension pulling on the inside.

If the dead cell contains, not water, but a solution, as at first it probably does, P_d is not zero and the equations require modification accordingly. An important difference between dead and living cells is then that the former (assuming the wall completely permeable to the solutes) will

not be turgid and the full osmotic pressure of its sap will be active, unbalanced by any turgor pressure. Equilibrium would require that $S_1 = P_a$.

Clearly, therefore, before the dead cell reaches a state of complete collapse the solutes must be completely removed.

Applying these considerations to the contractile root of *Brodiaea lactea*, we conclude that the progressive collapse of the yielding layers requires the progressive removal of their sap solutes. The withdrawal of water follows so long as the plant is not fully turgid. In so far as the tissues of the root are continuous and free from air-spaces, the pressure differences and, ultimately, cohesion tension will not act directly on the walls of the collapsing cells themselves but on the surfaces of the root (external or internal) in contact with the air. As DIXON has emphasized by reference to a model recently (1), a water deficit acts throughout the plant as if a reduced pressure or a tension were acting on all its surfaces, tending to compress the plant. Living cells withstand the pressure osmotically and their suction pressure is a measure of the compressing force they have to withstand. Dead cells, with permeable membranes allowing the escape of their solutes, or having yielded their solutes before death, can only avoid collapse in so far as their walls are rigid enough to withstand the compression or are protected by other structures taking the mechanical strain. In the case under consideration, the periderm and the stele are such structures offering resistance to longitudinal compression; and in proportion as the walls of the collapsing cells are weak, these tissues are left to bear the brunt of the compressing force.

The limits to the actual magnitude of this force in any particular case will depend upon the water balance of the plant when contraction is taking place. There is no reason to doubt its adequacy to produce all the deformations observed, seeing that it has generally to be measured in atmospheres. Although the experimental difficulties would be considerable, it should be possible to measure the force of contraction directly; but so far it seems that no one has made the attempt.

Summary

It is suggested that the explanation already offered of the mechanism of the contractile roots of *Oxalis incarnata* is also applicable to those of *Brodiaea lactea*. In both, transverse zones of cells collapse, and there is no tissue showing the continuity essential to an effective contractile tissue. The forces which bring about the ascent of sap are adequate to contract a root showing the features common to the roots of these two plants.

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BRIEF PAPERS

THE SENSITIVITY OF RED CLOVER (*TRIFOLIUM PRATENSE*) TO SMALL AMOUNTS OF BORON AND MANGANESE¹

(WITH ONE FIGURE)

In these brief notes attention is drawn to the extreme sensitivity of red clover (*Trifolium pratense*) to the omission of small amounts of boron and manganese where the plants are grown in distilled water culture solutions.

Many experiments of this nature have already been carried out by several workers both in this country and abroad. In many cases however depression in growth through lack of boron has not been obtained until the chemicals involved in the culture solution have been carefully purified so

TABLE I

DRY WEIGHTS OF RED CLOVER CULTURES GROWN IN SOLUTIONS TO WHICH VARYING
AMOUNTS OF BORON AND MANGANESE WERE ADDED

CULTURE SERIES	I		II		III	
TREATMENT	TOPS	ROOTS	TOPS	ROOTS	TOPS	ROOTS
	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>
Distilled water alone	0.706	0.196	0.870	0.350	0.617	0.183
Boron, <i>Ppm.</i>						
1.0	0.726	0.203	0.976	0.346	0.937	0.313
2.5	0.736	0.196	0.963	0.300	.	.
5.0	0.736	0.200	1.010	0.336	0.620	0.187
10.0	0.716	0.186	.	.	0.463	0.110
Manganese, 1.0	0.723	0.210	1.367	0.517
2.5	0.733	0.190
5.0	0.660	0.183	1.220	0.407
10.0	0.663	0.186	0.880	0.290
Boron, 1.0 } Manganese, 1.0 }	0.763	0.206	1.407	0.550
Boron, 2.5 } Manganese, 2.5 }	0.756	0.193
Boron, 5.0 } Manganese, 5.0 }	0.630	0.173	0.917	0.247
Tap water	0.776	0.230	1.206	0.408	1.090	0.347

¹ Contribution no. 408 of the Rhode Island Agricultural Experiment Station.

as to be practically boron-free. No such precautions were taken with the cultures here reported.

While carrying out experiments to determine the nutrient requirements of redbtop, Rhode Island bent grass, fescue and red clover, cultures were used with both tap and distilled water. With the grasses above mentioned, growth was uniformly good in both media. The growth of red clover, however, was distinctly depressed in the distilled water cultures. SOMMER and SOROKIN² have shown distinct morphological characteristics accompanying the lack of boron with *Pisum sativum*. As the gross root development was not normal the addition of small quantities of boron to the culture solution was indicated. Accompanying the depressed root development was a marked lighter color of leaves which might be considered as a form of incipient chlorosis. From previous work at this station and elsewhere a lack of manganese has been linked with chlorotic conditions. Boron was therefore added to the distilled water cultures in varying quantities, sodium



Distilled H ₂ O plus nutrient	1 ppm. boron plus nutrient	1 ppm. manganese plus nutrient	1 ppm. boron, 1 ppm. manganese plus nutrient	Tap H ₂ O plus nutrient
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FIG. 1. Effect on the growth in culture solution of red clover (*Trifolium pratense*) of the addition of small amounts of boron and manganese.

² SOMMER, A. L., and SOROKIN, HELEN. Effects of the absence of boron and of some other essential elements on the cell and tissue structure of the root tips of *Pisum sativum*. Plant Physiol. 3: 237-254. 1928.

borate being used. Manganese in the form of the sulphate was also supplied.

Table I gives the dry-weight yields of cultures with the various treatments used. The cultures were grown in triplicate and three series of cultures were grown at different times of the year. It will be noted that the yields of both tops and roots were increased by 1 part per million of boron; 2.5 parts per million of manganese, and a combination of boron 1 part per million and manganese 1 part per million. Greater amounts of these elements tended to depress growth. From figure 1 a visual comparison of the cultures may be obtained. It would therefore seem that the following two conclusions may be drawn.

1. When compared with certain grasses red clover, when grown in solution cultures, is much more sensitive to the absence of small amounts of boron and manganese.

2. Small amounts of boron and manganese when added to distilled water solutions tend to bring about increased root and top growth with red clover.—B. E. GILBERT AND F. R. PEMBER, *Rhode Island Agricultural Experiment Station*.

OBSERVATIONS ON THE RED COLOR OF THE BLOOD ORANGE

(WITH ONE FIGURE)

WHELDALE¹ makes the statement that anthocyanins are absent from citrus fruits "except in the red-fleshed variety, the so-called 'Blood Orange.'" She does not, however, give any experimental observations. The writer wishes to report the occurrence of the pigment in the fruit in crystalline form as well as to report some observations on the clear filtered juice.

The filtered juice has a pink to red color depending on the amount of pigment present. When acid (citric or hydrochloric) is added it turns to a deep pink or rose pink. The red pigment is not extracted from the juice by chloroform or ether. Ethyl acetate and amyl alcohol remove some of the red coloring matter. Sodium hydroxide changes the color to an emerald green. Lead carbonate and lead acetate produce a yellowish-green precipitate. The red color disappears when the juice is treated with zinc dust and hydrochloric acid but returns when the treated juice is allowed to stand.

Microscopic observation of the crushed juice sacs showed spherites or needle crystals of a deep red to reddish-brown color. One large group of crystals was blue-red, that is, about half way between a pure blue and a

¹ WHELDALE, M. The anthocyanin pigments of plants, footnote 1, p. 27, Cambridge, 1916.

pure red. A typical group of crystals is shown in the accompanying photomicrograph (fig. 1). Globules of red solution were also present, probably

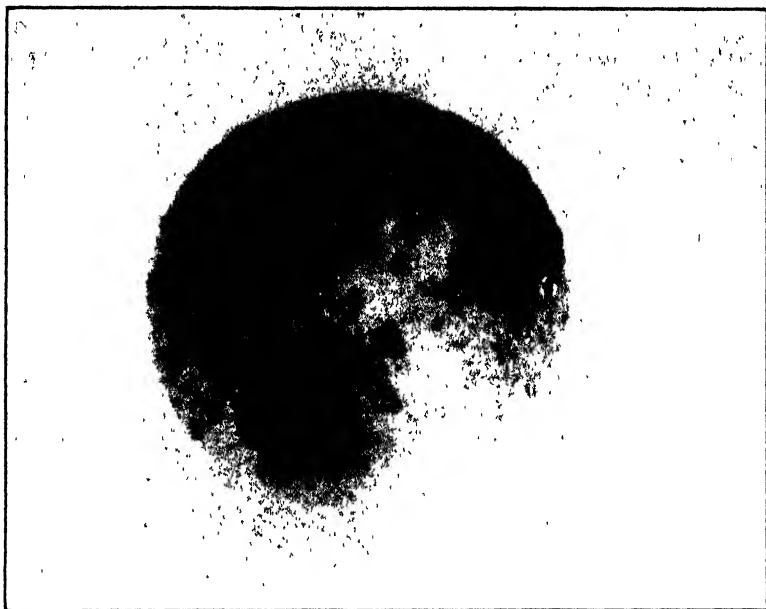


FIG. 1. Anthocyan crystals, $\times 110$.

held in separate cells of the juice sac. In one case long red needle-like crystals were found adjacent to a lump of deep red material. The red globules of solution and the crystalline material became clear blue when a drop of ammonium hydroxide was allowed to flow under the coverslip.—
M. B. MATLACK, *Food Research Division, Bureau of Chemistry and Soils, U. S. Department of Agriculture.*

NOTES

Annual Meeting.—The annual meeting of the American Society of Plant Physiologists at New Orleans during the week of December 28, 1931–January 2, 1932, should be largely attended. Meetings in the far south are rare. Not since 1904 has New Orleans been host to the scientific associations of America. Only twice during this long period have meetings been held south of Washington, D. C.: Atlanta in 1913, and Nashville, 1927. At Atlanta the weather was uncomfortably cold during the entire meeting. The Nashville meeting was memorable for the fine weather during the meetings, and the blizzard on the way home to all points north. New Orleans should provide an ideal setting for winter-time meetings, and visitors to that city will find much of historical, architectural, and social interest in addition to the scientific value of the meetings.

Members will have opportunity to offer papers to the program committee at an early date. Papers to be read by title only are not desirable for such meetings, and authors offering papers are urged to present them in person. A prompt response to requests for titles is always appreciated by the committee in charge of the program.

The headquarters for the meetings of the American Society of Plant Physiologists are in the St. Charles Hotel, New Orleans. Early reservation by those who are planning to attend will prevent disappointment in securing accommodations.

Life Membership Committee.—President Tottingham has named the committee for the CHARLES REID BARNES life membership award, which is to be made at New Orleans. The personnel of the committee is as follows: Dr. R. H. CARR, Purdue University, chairman; Dr. B. E. GILBERT, Rhode Island Agr. Exp. Sta.; Dr. E. M. HARVEY, U. S. Dept. Agr., Pomona, Calif.; Dr. W. D. KIMBROUGH, University of Louisiana; Dr. W. J. CRIST, Michigan State College. The announcement of the award has usually been made at the banquet held during the annual meeting.

Reprints.—The policy of PLANT PHYSIOLOGY with reference to free reprints may need clarification or at least restatement. In order to obtain free reprints the author must place an order for reprints for which he expects to pay. Against this order the first costs of making them up are assessed. In addition to this order, we then have made up fifty reprints without covers and include them in the shipment with no additional charge. If for instance an author wants 150 reprints without covers, he can place his order for 100, and will receive 150. If he places an order for 150, he will receive 200, etc. The author should take into consideration the reprints

furnished free when ordering. No reprints are made up if the author places no order for them.

Costs of Publication.—Members of the Society will be interested in the three items of cost involved in the production and distribution of the official journal, *PLANT PHYSIOLOGY*. Intelligent interest in these costs might assist in bringing about more economical publication; at least, certain items of cost could be reduced if members took an active interest in these matters. The figures for the January, 1931, and July, 1931 issues are as follows:

	January	July
Printing (including covers, and corrections)	940.58	939.87
Engravings	170.27	138.97
Distribution costs (postage, wrapping, stencils)	39.91	43.65
Total	\$1,150.76	\$1,122.49

These figures do not include the cost of separates presented to the authors of papers, but otherwise represent the entire cost to the Society of an edition of 1,000 copies of each number.

Specific attention is directed to the matter of corrections, included in the bill for printing costs. These cost from \$15.00 to \$40.00 per issue. The loss from this item cannot be eliminated entirely, but it can be reduced. It results mainly from the practice of certain authors who insist on re-writing their papers in galley proof. This is of course an absolutely illegitimate practice. The oversupply of papers from which nearly all journals suffer would be reduced somewhat if authors who take these illegitimate liberties would hold their papers long enough to perfect them before submitting them. In linotype printing, change of a letter involves resetting a whole line. Insertion of a word or phrase may require resetting of several lines, or a whole paragraph. For these changes the treasurer must pay. As the thoughtlessness of authors toward the problems of publication is so wide spread, we solicit earnest cooperation to stop the leaks in our printing costs.

Size of Manuscripts.—*PLANT PHYSIOLOGY* since March 1931 has had a definite surplus of publishable material. The editorial board has been averse to setting a size limit to papers offered for publication, but it is just inevitable that some policy must be adopted to control the size of the annual volumes of the journal. The American Society of Plant Physiologists is giving to its members and subscribers more literature per year than almost any other society of its age has ever offered. For example, no volume of the *American Journal of Botany* before 1929, the 16th volume of that journal, has contained as many pages as this, our 6th volume. Similar comparisons can be made with other journals, *Phytopathology*, *Ecology*, *Jour-*

nal of the American Society of Agronomy, etc. A very generous amount of space has been used, and yet it is impossible to publish acceptable manuscripts as fast as they are received.

To meet the situation a tentative method of control is being adopted which is intended first of all to discourage long papers, and secondly to control the size of the annual volume. We hope it will meet the approval of the general membership, and the editor would like an expression from a wide circle, either of approval or disapproval. The size of papers will not be limited arbitrarily, although size alone may determine the rejection of otherwise suitable papers. But the *number* of long papers (*i.e.*, exceeding 30 printed pages) used in each volume must be strictly limited for each volume. By experience in 1931 it has been found impossible to use more than three long papers during the year. The tentative proposal, therefore, is to include not more than one long paper in each of the first three issues of the year, and to make up the final number solely of shorter papers, and to limit the size of the volume to fit the income of the Society from memberships and subscriptions. The lengthy papers will be placed in a separate series from the shorter ones, and used in chronological order. The editor now has on hand about six of these long papers. Authors should guide themselves accordingly. Scrutiny of long papers reveals the fact that some of them could have been improved considerably by condensation. Comment on this proposed plan is desired by the editorial board, and will be gratefully considered.

Errata.—A number of errors have been detected in the pages of *PLANT PHYSIOLOGY* after publication. These have been listed at the close of the table of contents. We suggest that these errors be corrected at the places where they occur, to prevent future misinterpretation. The editor is grateful to the authors who assisted in preparing the list, and such cooperation is invited on the part of anyone finding mistakes in the journal.

Portraits.—A complete set of the 13 portraits published in *PLANT PHYSIOLOGY* during the last several years may be obtained for \$1.45, post-paid. In broken lots, send 12 cents in stamps for each portrait desired. They are suitable for offices, corridors, laboratories, libraries, etc., and stimulate interest in the historical development of science, particularly of plant physiology. Orders will receive prompt attention. Address the editor of *PLANT PHYSIOLOGY*, University of Chicago.

Principles of Plant Biochemistry.—This work is a publication from the University of Cambridge Press by Mrs. MURIEL WHELDALD ONSLOW, whose "Practical Plant Biochemistry," and "Anthocyanin Pigments of

Plants" are well known among plant physiologists. The book is devoted particularly to the chemistry of the carbohydrates and proteins, and to respiration. The two carbohydrate chapters consider the family of sugars, and the cell wall and its modifications. The third chapter takes up oxidizing and reducing systems, the fourth and fifth chapters deal with proteins of plants and nitrogen metabolism, and the final chapter with respiration. Eleven literature lists accompany the main sections, and provide about 975 references. While these do not properly reflect the American contributions to plant biochemistry, they offer a good summary of progress made in Europe. The book is obtainable for \$4.75, from the Macmillan Co., New York.

Alkaloids.—The second edition of G. TRIER'S great monograph, *Die Alkaloide*, has now been completed. The second volume has appeared this summer, the first having been printed in 1927. The volumes are paged consecutively, and contain 1,061 pages. The first 860 pages (*Spezieller Teil*) are occupied by accounts of I. the better known alkaloids, arranged in groups, aliphatic bases, aromatic bases, acid amides, urea derivatives, and heterocyclic bases of various types; II. the less well known bases found in cryptogams and phanerogams, arranged by families in the Engler-Prantl order; and III. chemically little known bases of general distribution (ptomaines, vitamins, hormones, etc.). The second section, pp. 861–984 (*Allgemeiner Teil*) deals with the problems of constitution and distribution of alkaloids, their biochemical and pharmacological significance, analysis and determination of constitution, etc. There is a supplement (*Nachtrage*), of 38 pages at the rear, a concluding discussion of tryptophane and the hypothesis of alkaloidal origin of blood- and leaf-pigments, an alphabetic list of the alkaloid-containing plant families (phanerogams only) and the index. The price, bound, is listed at 42 RM. Anyone desiring to order copies of this important monograph for personal or library use should have orders sent to Gebrüder Borntraeger, Schöneberger Ufer 12a, Berlin W 35, Germany.

Fruit Culture.—The trend of interest in scientific plant production the world over is toward a physiological foundation for cultural practice. A new text-book emphasizes this trend. It is FRITZ KOBEL'S *Lehrbuch des Obstbaus auf physiologischer Grundlage*, from the press of J. Springer, Berlin. This interesting book presents first the general physiology of fruit trees, intake of water, transpiration and water transport, absorption and utilization of mineral nutrients, photosynthesis, storage and use of reserves, effects of low and high temperatures, and vegetative growth.

The second section deals with blossom bud differentiation and growth, and the ways in which many environmental factors affect these processes.

Then the processes of fruit formation are considered, beginning with the opening of the flower, fertilization, and the normal fruit development following this process, etc. Such problems as sterility receive much attention, parthenocarp and apogamy are treated briefly, and the development of the fruit up to the ripe stage is depicted as a dynamic physiological process.

The last two sections deal with the relations between vegetative growth, fruit bud differentiation, and fruiting behavior, and with fruit breeding for new varieties. The book frankly accepts the carbohydrate-nitrogen relation hypothesis as the basis of explanation of much of the physiological behavior of fruit trees. It contains 274 pages of text, and is written in a style that will please those who are beginning to read and appreciate German scientific literature. It is recommended not only for its readability, but also for its solid value as a contribution toward physiological perspective in the plant sciences. The price is 16 RM. for unbound copies, or 18.4 RM. for cloth binding. Orders may be sent directly to Julius Springer, Linkstrasse 23-24, Berlin W 9, Germany.

Physical Properties of the Soil.—A summary of the available knowledge concerning the physical properties of the soil has been prepared by Dr. B. A. KEEN, soil physicist of the Rothamsted Experimental Station. It is one of the Longmans, Green and Co. series of Rothamsted Monographs on Agricultural Science. The opening chapter is an historical introduction presenting the relations between the development of knowledge of soil physics and the development of the arts and implements of soil culture. Chapter II considers the methods of physical soil analysis, and chapter III the physics of distribution and water movement in the soil. Succeeding chapters discuss the properties of soils of low moisture content, soil and clay pastes, soil and clay suspensions, soil constants and equilibrium points, and physical properties of soils under field conditions. The final chapters deal with soil temperature and soil aeration. The literature of soil physics is cited freely, and the bibliography at the close cites more than 250 papers, ample justice being done to contributions from this side of the Atlantic. Many footnotes cite other pertinent papers. The price of the book is \$8.00, and copies may be obtained from Longmans, Green and Co., New York.

Chemical Plant Physiology.—Dr. S. KOSTYCHEV's *Chemische Physiologie der Pflanzen*, which was published in a German edition in 1926, has been translated by Dr. C. J. LYON, of Dartmouth College. The value of a translation is usually determined by the accuracy of the translator, and the faithfulness with which the original work has been followed. In this case, however, KOSTYCHEV rewrote parts of the original especially

for this translated edition, and many changes will be found by one who compares the two. The chapter headings remain the same, and no changes have been made in the order of presentation. Editorial notes will be found among the foot-notes, with citations of literature pertinent to the discussion. Since many American students still do not make the effort to read German fluently, the translation will be helpful and time-saving. On general principles we do not believe in the translation vogue, and would prefer to see the originals in use by American students. The price of the work is \$6.00, and the publishers are P. Blakiston's Son and Co., Philadelphia.

Soil and Microbe.—An attractive account of the microbiology of soils has been written by Dr. S. A. WAKSMAN and R. L. STARKEY, of Rutgers University. It is entitled "The Soil and the Microbe." There are ten chapters, the first of which is concerned mainly with the processes of soil formation and plant nutrients. Chapters II and III discuss the microbes of the soil, their distribution and activities, and chapter IV the microbial decomposition of organic substances in the soil. The next three chapters deal with the transformations of soil nitrogen and mineral substances through the direct or indirect activity of microorganisms. Chapter VIII takes up the interrelations of higher plants and soil organisms, root excretions, mycorrhizas, nodule formation by legumes, bacteriorrhizas, etc. Chapter IX considers the modification of soil populations by cultural practices, and the last chapter summarizes the importance of microbes in soil fertility. A fairly comprehensive view of this field is presented, and the fundamental principles are set forth very clearly. The book is well illustrated, and many tables of data are included to elucidate the discussion. Each chapter closes with a small bibliography, but without periodic literature citations. The price of this useful book is \$3.50, and copies may be secured from the publishers, John Wiley and Sons, New York.

Handbook of Plant Nutrition.—The second volume of this work was noticed in the April, 1931 number of PLANT PHYSIOLOGY. We have now received the first volume, *Pflanzenernährung*, and it is a worthy companion to the preceding one. It contains 945 pages, and the ten chapters have the following titles: History of the development of plant nutrition; constituents and composition of the plant body; the cycle of substances in nature; the physiology of metabolism of plants; the soil as the habitat and nutrient reservoir for plants; the law of yield; water culture and growth experiments; field experiments; evaluation of fertilizer experiments; and determination of the nutritive deficiencies of the soil. Both volumes are well illustrated, and carry many literature citations at the ends of the various sections. It impresses one as a work of great value for scientific

workers, and for agriculturists who are interested in scientific plant culture. The editor of the volumes, Dr. F. HONCAMP, of Rostock, and the publishers, J. Springer, Linkstrasse No. 23-24, Berlin W 9, deserve the congratulations and thanks of all plant science investigators for this great contribution to plant nutrition and fertilizer science literature. The price of volume I unbound is RM. 93, bound RM. 96.8; and of volume II unbound is RM. 86, bound RM. 89.8. Orders may be sent directly to the publishers, in Berlin.

Plant Analysis.—The first volume of a four-volume *Handbuch der Pflanzenanalyse* has been published by J. Springer, I. Schottengasse 4, Vienna. The author, or editor, is G. KLEIN, each section being prepared by someone familiar with that particular phase of analytical work. This volume is mainly given over to general chemical and physical methods, rather than to systematic examination of plant tissues for their constituents. Such matters as the testing of reagents for purity, weighing, heating and cooling of materials, drying, recrystallization, filtering, centrifuging, dialysis, solubility separations, distillation and sublimation, occupy the first several chapters. Determination of elements, detection of radicals and groups by their characteristic reactions, gravimetric and volumetric procedures, histochemical methods, specific gravity, melting and boiling points, solubility, viscosity, and molecular weight determinations follow. Then come chapters which give methods for optical examination of materials, polarization, refractometry, interferometry, spectroscopy, spectrophotometry, colorimetry, etc., fluorometry, fluorescence, ultramicroscopy, and photochemical analysis. Other sections deal with electrical conductivity, electrometric and colorimetric determinations of hydrogen-ion concentration, and colorimetry. The closing sections deal with general procedures in chemical analyses, which will be continued in the second volume.

It is evident that the work covers a very wide range of methods, and that it will be a useful handbook for everyone who has to examine plant materials in any way whatever. The first volume contains 627 pages, and 323 figures. The price quoted for this volume is RM. 66 in paper cover, or RM. 69 bound in cloth. Orders for the complete work can be filed with the publisher, but the cost of the three unpublished volumes has not been stated. Libraries and research laboratories will find the work an indispensable aid in analytical work of all kinds.

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